# MYCOTAXON

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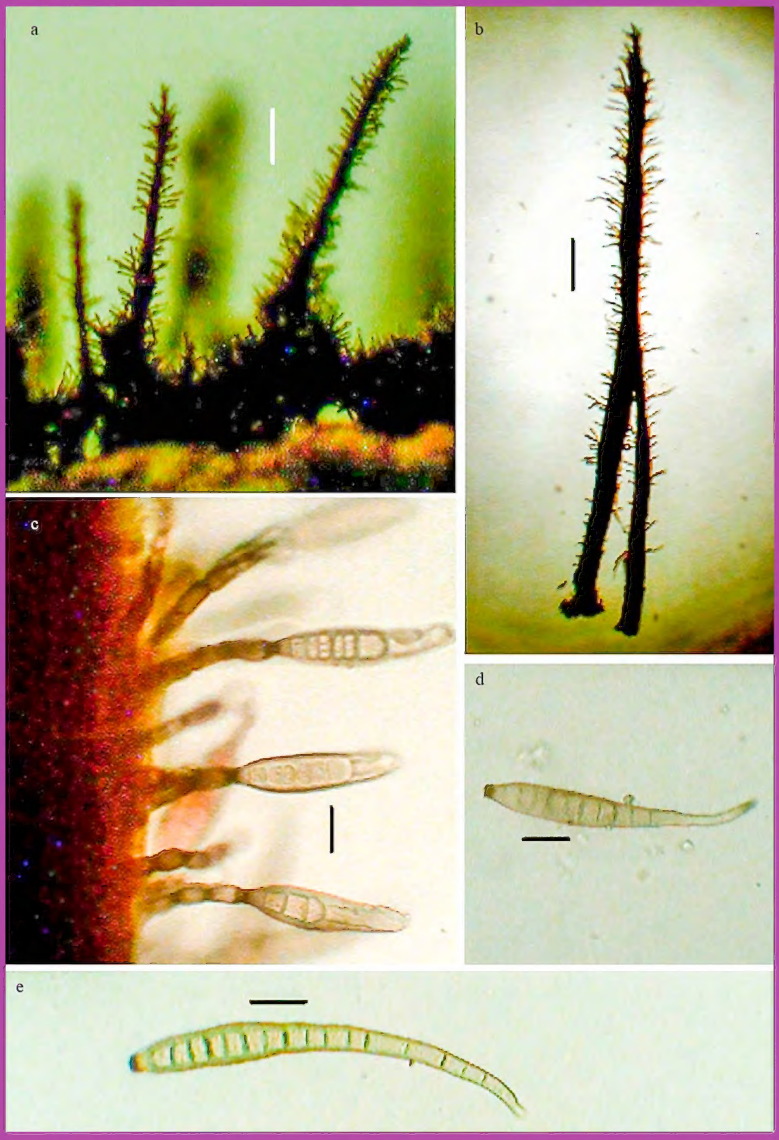
VOLUME 136 (3) JULY–SEPTEMBER 2021


*Neosporodesmium himachalense* sp. nov.
(Verma & al.— FIG. 2, p. 590)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# MYCOTAXON

VOLUME ONE HUNDRED THIRTY-SIX (3) — TABLE OF CONTENTS

*Reviewers* ..... vi
*Nomenclatural novelties & typifications* ..... vii
*Corrigenda* ..... viii
*From the Editor* ..... ix
*2021 submission procedure* ..... xii

New taxa

*Vanakripa chinensis* sp. nov. from China and notes on the genus — Kai Zhang, Weihua Guo, De-Wei Li, Rafael F. Castañeda-Ruiz 545

*Cordana sinensis* sp. nov. from southern China — Ling Qiu, Kai Zhang, Zhao-Huan Xu, Rafael F. Castañeda-Ruíz, Jian Ma 553

*Pezicula endophytica* sp. nov., endophytic in *Dendrobium* from Thailand — Xiao-Ya Ma, Ji-Chuan Kang, Kevin D. Hyde, Mingkwan Doilom, Putarak Chomnunti 563

*Globoramichloridium delicatum* and *Heteroconium simile* spp. nov. from southern China — Ling Qiu, Xu-Gen Shi, Xiu-Guo Zhang, Rafael F. Castañeda-Ruíz, Ji-Wen Xia, Jian Ma 579

*Neosporidesmium himachalense* sp. nov. from India and *Neosporidesmina* gen. nov. to accommodate *N. micheliae* — Rajnish Kumar Verma, I.B. Prasher, Sushma, Ajay Kumar Gautam, Kunhiraman C. Rajeshkumar, Ashish Kumar, Rafael F. Castañeda-Ruiz 587

*Cladorrhinum yunnanense* sp. nov. from China — Yue Pan, Ji-wen Xia, Sheng-ting Huang, Chun-yuan Zhu, Xiu-guo Zhang, Zhuang Li 597

*Mazosia hainanensis* sp. nov. from tropical China — Zong-Ting Yao, Ze-Feng Jia, Shu-Hua Jiang 607

*Racocetra cromosomica* sp. nov. from Oaxaca, Mexico — Eduardo Chimal-Sánchez, Irma Reyes-Jaramillo, Sara Lucía Camargo-Ricalde, Lucía Varela, Jose Yabín Salmerón Castro, Noé Manuel Montaño 615

*Bactrospora cozumelensis* sp. nov. from Mexico Yenitze A. García-Martínez, Jorge Guzmán-Guillermo, Ricardo Valenzuela, Tania Raymundo 627

*Lembosia mimusopis* sp. nov. from Thailand Diana S. Marasinghe, Monika C. Dayarathne, Sajeewa S.N. Maharachchikumbura, Abdallah M. Elgorban, Sinang Hongsanan, Kevin D. Hyde 635

Environmental range verification

*Fibroporia gossypium* isolated from an indoor environment in Argentina Carolina A. Robles, María Belén Pildain, Francisco Sautua, Marcelo A. Carmona, Mario Rajchenberg 645

New ranges/hosts

*Rubellofomes cystidiatus* and *Spongiporus floriformis* newly recorded for India Vinjusha Nellipunath & T.K. Arun. Kumar 661

*Diplodia bulgarica,* a new record for Turkey Cafer Eken 669

*Gerhardtia foliicola*, a new record for Pakistan Aiman Izhar, Muhammad Usman, Munazza Kiran, Abdul Nasir Khalid 675

Mycobiota (fungae) new to the Mycotaxon website

Checklist of the lichens of The Reserva Florestal Adolphe Ducke in Manaus (Amazonas, Brazil) (summary) André Aptroot, Janice Gomes Cavalcante, Lidiane Alves dos Santos, Isaias Oliveira Junior, Dayane de Oliveira Lima, Marcela Eugenia da Silva Cáceres 685

First molecular-based contribution to the checklist of Lebanon macrofungi (summary) Sandra Sleiman, Jean-Michel Bellanger, Franck Richard, Jean Stephan. 687

Checklist of powdery mildew fungi (*Erysiphaceae*) of Pakistan (summary) N.S. Afshan, I. Zafar, A.N. Khalid 689

Aphyllophoroid fungi (*Basidiomycota*) of Chile: An annotated checklist (summary) Cristian Riquelme & Mario Rajchenberg 691

## Reviewers — volume one hundred thirty-six (3)

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this issue.

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## Nomenclatural novelties and typifications proposed in Mycotaxon 136(3)

*Bactrospora cozumelensis* Y. García, Guzm.-Guill., R. Valenz. & Raymundo
[MB 836514], p. 630

*Cladorrhinum yunnanense* Y. Pan, J.W. Xia, X.G. Zhang & Z. Li
[MB 836106], p. 602

*Cordana sinensis* L. Qiu, K. Zhang, R.F. Castañeda & Jian Ma
[MB 836854], p. 556

*Globoramichloridium delicatum* L. Qiu, Jian Ma, R.F. Castañeda & X.G. Zhang
[MB 836851], p. 580

*Heteroconium simile* L. Qiu, Jian Ma, R.F. Castañeda & X.G. Zhang
[MB 836852], p. 582

*Lembosia mimusopis* Marasinghe, Daya., Maharachch., Hongsanan & K.D. Hyde,
[IF 556875], p. 640

*Mazosia hainanensis* Z.T. Yao, S.H. Jiang & Z.F. Jia
[MB 836195], p. 610

*Neosporidesmina* R.F. Castañeda, Rajn.K. Verma, Prasher, Sushma, A.K. Gautam & Rajeshk.
[IF 557995], p. 592

*Neosporidesmina micheliae* (Y.D. Zhang & X.G. Zhang) R.F. Castañeda, Rajn.K. Verma, Prasher, Sushma, A.K. Gautam & Rajeshk.
[IF 557996], p. 592

*Neosporidesmium himachalense* Rajn.K. Verma, Prasher, Sushma, A.K. Gautam, Rajeshk. & R.F. Castañeda
[IF 557994], p. 589

*Pezicula endophytica* X.Y. Ma, K.D. Hyde & J.C. Kang
[IF 558634], p. 570

*Racocetra cromosomica* Chim.-Sánch., Varela & Montaño
[IF 557326], p. 618

*Vanakripa chinensis* K. Zhang, W.H. Guo, R.F. Castañeda & D.W. Li
[IF 557829], p. 546

# Corrigenda

## Mycotaxon 136(2)

p. 343, line 14 for: Basionym: R.H. Petersen & K.W. Hughes. 2014. N. Am. Fungi 9: 6.
read: Basionym: *Gymnopus micromphaloides* R.H. Petersen & K.W. Hughes. 2014. N. Am. Fungi 9: 6.
note: Omission of the basionym name invalidated the proposed combination. *Collybiopsis micromphaloides* was validly published in Petersen & Hughes, Index Fungorum 491: 1 (2021).

pp. 359–360 Missing! The missing pages resulting from misnumbering p. 361 were not noticed until after publication

publication date for volume one hundred thirty-six (2)

Mycotaxon *for* April–June 2021 (i–xii + 263–544)
*was issued on July 23, 2021*

## From the Editor-in-Chief

So, you think you've found a new species? One of the joys your Mycotaxon editors share is overseeing the publication of new taxa (the primary focus of this journal) and which generally involves shepherding authors to the realization that a new species best withstands the scrutiny of other researchers and the test of time if it is based on more than a single specimen. Our *Nomenclature Editor* shares his thoughts on the subject:

> "The ideal standard for a proposal of a new taxon: Many manuscripts submitted to Mycotaxon propose a new taxon based on a single specimen (the holotype). This is completely acceptable if this is the only known material, but it has come to our attention that some of these manuscripts are failing to report other specimens that have been collected and vouchered. Whenever possible, the proposal of a new taxon should present: (1) a type collection that was large enough to be divided and vouchered in two (or more) different herbariums, one designated as the **holotype** and the others as **isotypes**; (2) other specimens (ideally collected from different localities, or from different hosts/substrates, or on different dates), which the manuscript should present, with full collection and vouchering data, in an Additional specimen(s) examined section inserted immediately after the description of the new taxon. Authors should aim to achieve this ideal standard when collecting material and are encouraged (one might even say required) to report all conserved specimens of a new taxon in any manuscript inaugurating a new taxon."

Contending with only a single specimen on which to base a new species (and possibly new genus) has long been a bane of careful workers. With the advent of sequence analyses, many have been driven to "check the DNA" to reassure themselves that their morphologically and/or culturally based holotype also differs phylogenetically. Unfortunately, several isolates obtained from a single specimen do not increase species support when they cluster together nor do they address the variation likely to be encountered in different specimens. Both phylogenetic and morphological species concepts are strengthened when several specimens are studied.

Of paratypes and protologues. Early in my taxonomic studies, I was convinced that a collection cited by a scientist in the examined specimens sections of a new species (technically referred to as a paratype) had taxonomic and nomenclatural significance. Not until I compared several paratype collections with one holotype specimen to discover several cryptic but morphologically distinct species hidden under one name did I realize that while a paratype (representing the original author's species concept) should always be evaluated as taxonomically important, it held no nomenclatural significance, despite its "-type" suffix. A paratype is simply a specimen

included in the protologue of the type description. And the PROTOLOGUE? As defined in the DICTIONARY OF THE FUNGI (Kirk & al. 2008), it is: "everything associated with a name on its first publication, i.e. diagnosis, description, references, synonymy, geographical data, citation of specimens, discussion, comments, illustrations."

The delayed 2021 July–September MYCOTAXON, is unusually slim, offering 18 contributions by 79 authors (representing 14 countries) as revised by 40 expert reviewers and the editors.

With ten titles, the NEW TAXA section proposes ONE new genus (*Neosporidesmina* from India) plus 11 species new to science representing *Bactrospora* and *Racocetra* from MEXICO; *Cladorrhinum, Cordana, Globoramichloridium, Heteroconium, Mazosia, Pezicula,* and *Vanakripa* from CHINA; *Lembosia* from THAILAND; and *Neosporidesmium* from INDIA. We also offer one new combination in *Neosporidesmina* from India.

The single title in the ENVIRONMENTAL RANGE VERIFICATION section explores the mysterious discovery of *Fibroporia gossypium,* a widely distributed brown-rot polypore (described 122 years ago by Spegazzini and normally collected in conifer (Europe, North America, Asia) or *Nothofagus* forests) in an abandoned basement of a university plant pathology lab.

The NEW RANGES/HOSTS section contains three titles reporting range extensions for [basidiomycetes] *Rubellofomes* and *Spongiporus* for INDIA and *Gerhardtia* for PAKISTAN and [dothideomycetes] *Diplodia* for TURKEY. MYCOTAXON 136(3) also provides keys to species in *Cordana, Mazosia,* and *Vanakripa,* establishes a new *Racocetra* species through culture study, and confirms the pathogenicity of *Diplodia bulgarica* on apples in Turkey.

Papers providing conclusions supported by sequence analyses cover six new species representing *Cordana, Cladorrhinum, Lembosia, Mazosia, Pezicula,* and *Vanakripa* and five environmental or range extensions for *Diplodia, Fibroporia, Gerhardtia, Rubellofomes,* and *Spongiporus.*

Our issue concludes with the announcement of four excellent new annotated species lists on our MYCOBIOTA website, covering lichens in BRAZIL, macrofungi of LEBANON, powdery mildews of PAKISTAN, and the aphyllophoroid fungi of CHILE.

Warm regards and best wishes,
Lorelei L. Norvell (*Editor-in-Chief*)
Shaun R. Pennycook (*Nomenclature Editor*)
15 October 2021

## 2021 Mycotaxon submission procedure

Prospective Mycotaxon authors should download the Mycotaxon 2021 guide, review & submission forms, and Mycotaxon sample manuscript by clicking the 'file download page' link on our Instructions to Authors page before preparing their manuscript. This page briefly summarizes our '4-step' submission process.

1—Peer review: Authors first contact peer reviewers (two for journal papers; three for mycobiota/fungae) before sending them formatted text & illustration files and the appropriate 2021 Mycotaxon journal or mycota reviewer comment form. Experts return revisions & comments to both the *Editor-in-Chief* <editor@mycotaxon.com> and authors. All co-authors must correct and *proof-read* their files before submitting them to the *Nomenclature Editor*.

2—Nomenclatural review: Authors email all **error-free** text & illustration files to the *Nomenclature Editor* <PennycookS@LandcareResearch.co.nz>. Place **first author surname** + **genus** + 'Mycotaxon' on the subject line, and (required) attach a completed submission form. The Nomenclature Editor will (i) immediately assign the accession number and (ii) after a few weeks return his notes and suggested revisions to the author(s) and *Editor-in-Chief*.

3—Final submission: All coauthors thoroughly revise and proof-read files to prepare error-free text and images ready for immediate publication. Poorly formatted copy will be rejected or returned for revision. E-mail the final manuscript to the *Editor-in-Chief* <editor@mycotaxon.com>, adding the **accession number** to the message and **all** files, which include a **(i)** revised 2021 submission form, all **(ii)** text files and **(iii)** jpg images, and **(iv)** FN, IF, or MB identifier verifications for each new name or typification. The *Editor-in-Chief* acknowledges submissions within two weeks of final submission but requests authors to wait at least 14 days before sending a follow-up query (without attachments).

4—Final editorial review & publication: The *Editor-in-Chief* conducts a final grammatical and scientific review and returns her editorial revisions to all expert reviewers and coauthors for final author approval. Author-approved files are placed in the publication queue.

The PDF proof and bibliographic & nomenclatural index entries are sent to all coauthors for final inspection. After PDF processing, the *Editor-in-Chief* corrects only PDF editorial/conversion and index entry errors; corrections of all other errors are listed in the Errata of a subsequent issue for no charge. Authors will pay fees for mycobiota uploads, optional open access, and correction of major author errors to the *Business Manager* <subscriptions@mycotaxon.com> at this time.

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# *Vanakripa chinensis* sp. nov. from China and notes on the genus

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Abstract—A new species *Vanakripa chinensis* found on decaying branches of an unidentified tree is described and illustrated. It is characterized by sporodochial conidiomata composed of macronematous, cylindrical conidiophores that are sometimes reduced to conidiogenous cells and obovoid, unicellular, brown, smooth conidia with a black spot near the middle. Notes on conidiogenous events in the genus *Vanakripa* and a key to the species are provided.

Key words—asexual fungi, hyphomycetes, taxonomy

## Introduction

*Vanakripa,* was introduced by Bhat & al. (Bhat & Kendrick 1993) with *V. gigaspora* as type species; it is distinguished by scattered, punctiform, pulvinate to postulate black or dark brown loosely or compact sporodochial conidiomata. The conidiophores are macronematous, mononematous, cylindrical below and inflated toward the apex, erect, flexuous, un- or slightly branched, refractively septate, smooth, and hyaline. The conidiogenous cells are monoblastic, cylindrical to narrowly or broadly clavate, truncated after

conidial secession, terminal, integrated, hyaline (sometimes with a refractive lumen), and smooth. Conidia are solitary, acrogenous, variably shaped (clavate, broadly pyriform, obovoid, ellipsoidal), truncate at the base and rounded at the apex, 0–1-septate, darkly pigmented, smooth, thick walled, usually remaining attached to the conidiogenous cells (Bhat & Kendrick 1993). Under the predominantly subtropical climate of Yunnan province, several novel asexual fungi have been found in the rainforest and mountains by Guo & al. (2019), Zhang & al. (2020), and Zheng & al. (2019, 2020a,b). During a survey of fungal diversity in Xishuangbanna, Yunnan province, an interesting fungus was collected on decaying branches; its conidiomata, conidiogenesis, and conidial features clearly suggest a placement in the genus *Vanakripa*.

## Materials & methods

Samples of decaying plants in plastic were bags placed and transported to the laboratory, placed in moist chambers, and treated according to Castañeda-Ruiz & al. (2016). Mounts were prepared in lactic acid (90%) or in polyvinyl alcohol-glycerol (8 g PVA in 100 mL of water + 5 mL of glycerol). Microscopic characters were measured at ×1000 using a Nikon Eclipse Ni-U microscope with DIC optics and a Nikon DS-Fi2 camera. The holotype was deposited in the Herbarium of Department of Plant Pathology, Shandong Agricultural University, China (HSAUP).

## Taxonomy

***Vanakripa chinensis*** K. Zhang, W.H. Guo, R.F. Castañeda & D.W. Li, **sp. nov.** Fig. 1

IF 557829

Differs from *Vanakripa fasciata* by its narrow obovoid, smaller conidia; and from *V. rhizophorae* by its smaller, uniformly obovoid, dark spotted conidia.

Type: China. Xishuangbanna Tropical Botanical Garden, on decaying branches of an unidentified broadleaf tree, 22 July 2019, coll. K. Zhang & W.H. Guo (**Holotype**, HSAUP Y190114).

Etymology: Latin, *chinensis,* referred to the country of collection, China.

Colonies on the natural substrate effuse, black. Conidiomata sporodochial, scattered, punctiform, pulvinate or pustuliform, ≤250 µm diam. Mycelium superficial and immersed, composed of septate, branched, hyaline, smooth-walled hyphae. Conidiophores macronematous, mononematous, erect, straight or flexuous, 0–2-septate, sometimes refractive at the septa, smooth-walled, hyaline, ≤35 µm length, sometimes reduced to conidiogenous cells. Conidiogenous cells monoblastic, cylindrical, sometimes narrowly clavate lumen refractive or translucent, determinate, integrated, terminal, smooth, hyaline, 15–29 × 2.5–5 µm. Conidial secession schizolytic. Conidia solitary,

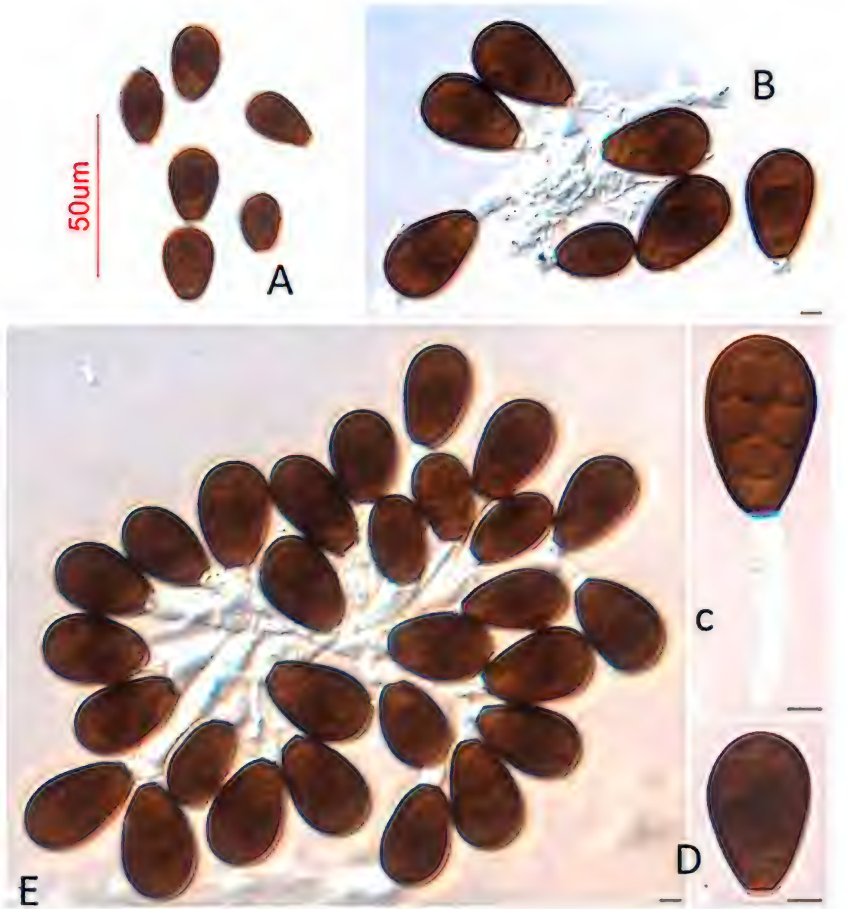


Fig. 1. *Vanakripa chinensis* (holotype, HSAUP Y190114). A. Conidia after schizolytic secession; B. Detached and attached conidia and conidiogenous cells; C. Conidiogenous cell with attached conidium; D. Conidium after schizolytic secession; E. Sporodochium. Scale bars: B–E = 5 μm.

acrogenous, obovoid to broad obovoid, truncate at the base, unicellular, thick-walled, guttulate, 19.8–22.8 × 12.7–14.8 μm, smooth, brown to black, with a conspicuous or inconspicuous black spot near the middle.

Notes: *Vanakripa* now comprises nine accepted species (Index Fungorum 2020; this paper): *V. chinensis*, *V. fasciata* R.F. Castañeda & al., *V. gigaspora* Bhat & al., *V. inexpectata* S.M. Leão & Gusmão, *V. inflata* (Hol.-Jech.) Melnik, *V. menglensis* D.M. Hu & al., *V. minutiellipsoidea* Pinnoi, *V. parva* Bhat & al.,

and *V. rhizophorae* R.M. Arias & al. ϟArias-Mota & al. 2008, Bhat & Kendrick 1993, Castañeda-Ruiz & al. 2005, Hu & al. 2010, Leão-Ferreira & al. 2013, Mel'nik 2011, Pinone & al. 2003). Additionally there is an invalid species, "*V. ellipsoidea*" K.M. Tsui & al. (Tsui & al. 2003), which lacks a designated holotype. *Vanakripa chinensis* superficially resembles *V. fasciata* in the dark spotted conidia, but *V. fasciata* has bigger (24–34 × 16–23 µm) ellipsoidal conidia that are dark brown with a black transverse band at the centre. Also somewhat similar to *V. sinensis, V. rhizophorae* is distinguished by its brown to black, variably shaped (globose, ovoid to ellipsoidal; 16.6–22.3 × 6.3–14.7 µm) conidia.

**Key to *Vanakripa* species**

1. Conidia one-celled ..... 3
1. Conidia with a submedian septum ..... 2
2. Conidia ellipsoidal or obovoid, 17–27 × 7–12 µm, smooth, asymmetric, apical cells large, brown to dark brown, basal cells small, pale brown ..... *V. inexpectata*
2. Conidia clavate, obovoid to obpyriform, 32–43 × 18–22 µm, smooth, asymmetric, apical cells large, 30–38 µm long, dark brown to black, basal cell small, 3–5 µm long, pale brown ..... *V. gigaspora*
3. Conidia mostly ovoid, obovoid, or fusiform, truncate at the base ..... 4
3. Conidia not primarily ovoid, obovoid, or fusiform ..... 5
4. Conidia obovoid, larger (19.8–22.8 × 12.7–14.8 µm), guttulate, brown to black, with a conspicuous or inconspicuous black spot near the middle, smooth ..... *V. chinensis*
4. Conidia ovoid or fusiform, smaller (7–10 × 4–6 µm), brown to dark brown ..... *V. parva*
5. Conidia mostly ellipsoidal ..... 6
5. Conidia mostly clavate or globose ..... 8
6. Conidia fasciate, ellipsoidal, 24–34 × 16–23 µm, brown to dark brown, with a black transverse band at the centre, smooth-walled ..... *V. fasciata*
6. Conidia not fasciate ..... 7
7. Conidia ellipsoidal, 25–33 × 16–23 µm, with rounded apex and base, dark brown to black, smooth-walled ..... *V. minutiellipsoidea*
7. Conidia broadly ellipsoidal to subspherical, 14.5–24 × 9.5–19.5 µm, truncate at the base, dark brown, smooth-walled; conidiogenous cell 27–48 × 8–14 µm ..... *V. inflata*
8. Conidia clavate to obpyriform, truncate at the base, (8–)17–23 × 8–13 µm, brown to dark brown, smooth; conidiogenous cells 20–40 × 4–6 µm ..... *V. menglensis*
8. Conidia globose, obovoid to ellipsoidal, 17–22 × 6.3–14.7 µm, dark brown to black, smooth; conidiogenous cells 29.4–54 × 4–9.3 µm ..... *V. rhizophorae*

## Discussion

Bhat & Kendrick (1993) described *Vanakripa* as having "conidiogenous cells [that are] holoblastic, obpyriform, clavate, colorless, often curved, narrowed toward the base, truncate after conidial secession" and "conidia [that are] clavate to obpyriform, rounded at the tip, truncate at the base, darkly pigmented, smooth, 0–1-septate." Obviously, the conidial secession of *Vanakripa* is schizolytic, and only after this event, both conidiogenous cells and conidia have flat or truncate scars at the cell apex and conidial base. A conidiogenous cell is defined as any cell from or within which a conidium is directly produced, and a locus is the place on the conidiogenous cell where a conidium arises (Kendrick 1971, Kirk & al. 2008). In *Vanakripa* mature conidia usually remain attached on the conidiogenous loci, and after mechanical disturbance the conidiogenous cells and conidia separate by rhexolytic secession from the conidiophores. However, conidia that have been captured from the surface of substrate or released from the conidiogenous cells in a drop of KOH 4% show a flat, truncate base without any portion or frill of the conidiogenous cells (Fig. 1A). Bhat & Kendrick (1993), Holubová-Jechová (1983), Leão-Ferreira & al. (2013), Hu & al. (2010), and Seifert & al. (2011), have illustrated similar flat, basal truncated conidia. Hughes (1951) discussed Penzig's illustration of *Beltrania rhombica* Penz., with special reference to the attached intermediate cell found between conidiogenous cell and conidia that Penzig called "stermmi" to be later renamed a "separating cell" by Hughes (1951) and also adopted by Pirozynski (1963) in his taxonomical studies of *Beltrania*, *Beltraniella*, *Beltraniopsis*, and *Ellisiopsis*. In the strict sense, a separating cell is a cell between a conidium and a conidiogenous cell (Kirk & al. 2008); but the term is also applicable to the conidium, at first attached to the conidiogenous cell, then separating by schizolytic or rhexolytic secession. In *Vanakripa* the conidiogenous cells are monoblastic, terminal, integrated, cylindrical, vermiform, long clavate, usually slightly or strongly inflated near the conidiogenous locus and the conidia are produced on the apical locus, but not separating cell, which exists between the conidiogenous cells and conidia (Bhat & Kendrick 1993). However, separating cells associated with rhexolytic and schizolytic conidial secession have also been reported and illustrated in *Pseudotrichoconis*, *Rhexodenticula*, and *Dictyoaquaphila* (Baker & al. 2001, Li & al. 2011, Arias-Mota & al. 2016, Monteiro & al. 2016).

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# *Cordana sinensis* sp. nov. from southern China

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Abstract—A new species, *Cordana sinensis*, is described and illustrated from a specimen collected on dead branches of an unidentified broadleaf tree in Jiangxi Province, China. It is characterized by its integrated, polyblastic conidiogenous cells that produce ovoid to obclavate, medially 1–2-septate pale brown, smooth conidia with a slightly prominent hilum. A dichotomous key and a synoptic table to *Cordana* species, are provided.

Key words—asexual *Ascomycota*, *Cordanaceae*, hyphomycetes, saprobes, taxonomy

## Introduction

*Cordana* Preuss was established with *C. pauciseptata* Preuss as the type species (Preuss 1851) and characterized mainly by macronematous, mononematous, unbranched, cylindrical, nodose or subnodose conidiophores and polyblastic, integrated, terminal or intercalary, sympodial conidiogenous cells, usually denticulate with tiny or conspicuous cylindrical denticles. The conidia are solitary, acropleurogenous, variably shaped (ovoid, obovoid, ellipsoidal, pyriform, or obpyriform), 0–1-euseptate and seceding schizolytically (Preuss 1851, Hughes 1955, Ellis 1971, Markovskaja 2003,

Seifert & al. 2011). Hernández-Restrepo & al. (2014), who expanded the generic concept to include integrate and discrete polyblastic conidiogenous cells that are intercalary nodose and swollen or umbellate at the conidiophore apices, provided a key for 19 accepted species. Thereafter, *C. johnstonii* M.B. Ellis, *C. musae* (Zimm.) Höhn., and *C. versicolor* D.J. Soares & R.W. Barreto were transferred to *Neocordana* Hern.-Rest. & Crous based on a phylogenetic analysis (Hernández-Restrepo & al. 2015). After the addition of four *Cordana* species by Ai & al. (2019) and Luo & al. (2019), *Cordana* currently contains 20 recognized species, of which only ten have been reported from China: *C. abramovii* Seman & Davydkina, *C. aquatica* Z.L. Luo & al., *C. ellipsoidea* de Hoog, *C. lignicola* Z.L. Luo & al., *C. lithuanica* Markovsk., *C. lushanensis* C.C. Ai & al., *C. meilingensis* C.C. Ai & al., *C. pauciseptata*, *C. uniseptata* L. Cai & al., and *C. terrestris* (Timonin) Hern.-Restr. & al. (Lu & al. 2000, Cai & al. 2004, Zhang 2018, Ai & al. 2019, Luo & al. 2019).

In the past, *Cordana* was associated with *Trichosphaeriaceae* (Müller & Samuels 1982) and *Chaetosphaeriaceae* (Réblová & al. 1999) based on ascomatal morphology.

Saprobic hyphomycetes are highly diverse on dead branches, and much taxonomic information has been published in China (e.g., Wu & Zhuang 2005; Ma & al. 2014, 2015, 2016; Ma & Zhang 2015; Xia & al. 2015, 2016; Ma 2016a,b; Xu & al. 2017, 2019; Zhang 2018; Qiu & al. 2020). In our study of these fungi from Jinggangshan Mountain, Jiangxi Province, an interesting hyphomycete with the morphological features typical of *Cordana* was collected on dead branches. It shows significant differences from previously described *Cordana* species and therefore is proposed as new.

## Materials & methods

Dead branches were collected from humid environments and watersides in the forest ecosystems of southern China and placed in Ziploc™ plastic bags for transport to the laboratory, where they were processed, and examined as described by Ma & al. (2011). Microphotographs were prepared using a Nikon Eclipse E200 and SmartV550Dc digital camera, with a 100 × (oil immersion) objective at the same background and scale. Conidia were measured at their widest point. The minimum and maximum values for microscopic measurements are provided, with outliers in parentheses. Adobe Photoshop 7.0 was used to assemble photographs into plates. The specimens are deposited in the Herbarium of Jiangxi Agricultural University, Plant Pathology, Nanchang, Jiangxi, China (HJAUP).

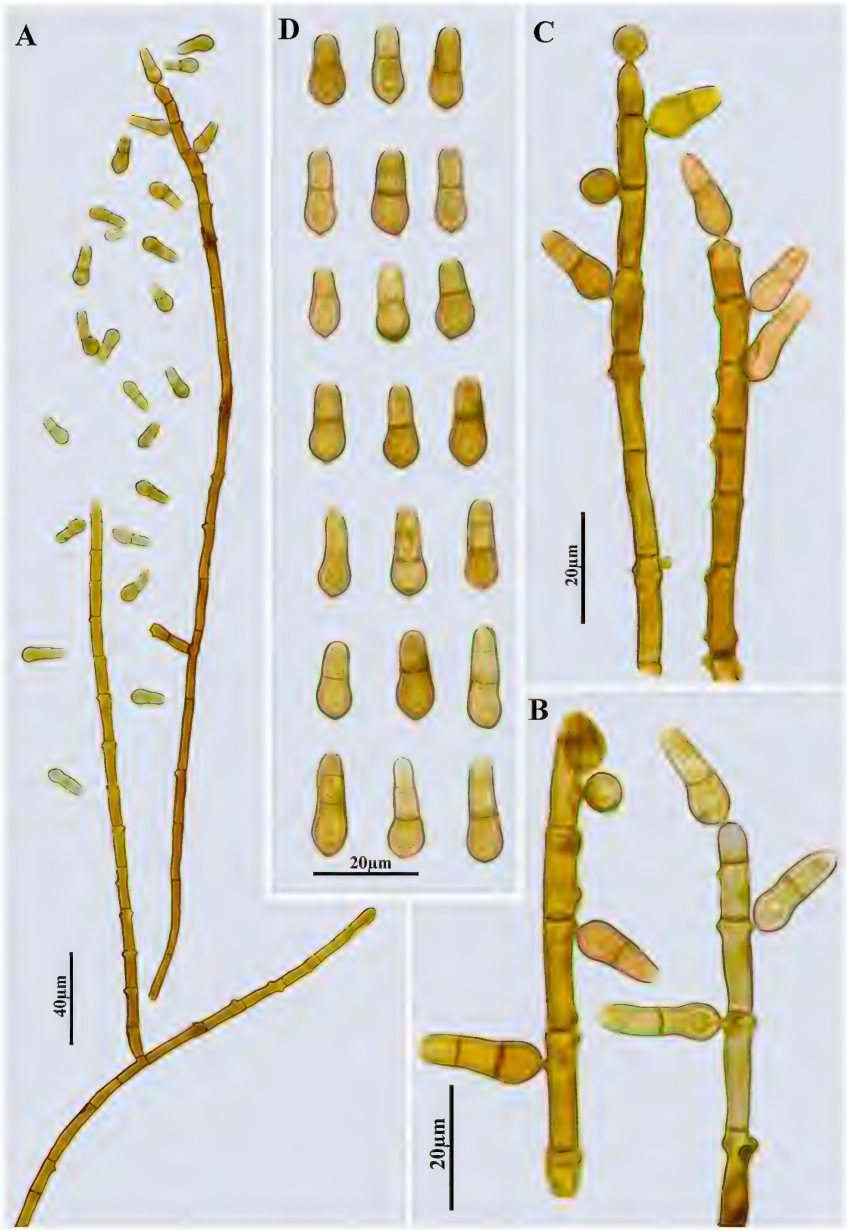


Fig. 1. *Cordana sinensis* (holotype, HJAUP M0172).
A–C. Conidiophores and conidia; D. Conidia.

## Taxonomy

***Cordana sinensis*** L. Qiu, K. Zhang, R.F. Castañeda & Jian Ma, **sp. nov.** FIG. 1

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Differs from *Cordana crassa* and *C. lithuanica* by its ovoid to obclavate, medially 1-septate or with 2-septate, narrower conidia.

TYPE: China, Jiangxi Province: Jinggangshan Mountain, Jingzhushan scenic spot, 26°32′28″N 114°06′57″E, on dead branches of an unidentified broadleaf tree, 6 Nov. 2014, J. Ma (**holotype**, HJAUP M0172).

ETYMOLOGY: refers to China, where the fungus was collected.

COLONIES on the natural substratum effuse, brown to dark brown. Mycelium partly superficial, partly immersed, composed of branched, septate, smooth, subhyaline to pale brown hyphae. CONIDIOPHORES macronematous, mononematous, simple or branched, erect, straight to flexuous, smooth, septate, brown to pale brown, cylindrical, sub-nodose, ≤400 µm long, 4–6.5 µm wide, with terminal and intercalary nodes, 5–7.5 µm wide. CONIDIOGENOUS CELLS integrated, polyblastic, terminal and intercalary, cylindrical-subnodose, with tiny prominent denticles at the nodes, percurrent elongated, 9.5–32 × 4–6.5 µm. Conidial secession schizolytic. CONIDIA solitary, acropleurogenous, ovoid to obclavate, 1–2-septate, pale brown, smooth, thick-walled, 13–20(–26.5) × 5–7.5 µm, dry, apical cell rounded, basal cell with a tiny prominent hilum.

COMMENTS –Among the known species of *Cordana*, *C. sinensis* most closely resembles *C. crassa* Tóth and *C. lithuanica* Markovsk. in conidial shape. However, *C. crassa* and *C. lithuanica* produce asymmetrically 1-septate conidia while those of *C. sinensis* are medially 1–2-septate. In addition, *C. crassa* produces larger (20–24 µm long), ovoid conidia (Tóth 1975), and *C. lithuanica* produces shorter and wider (13–17.5 × 8–10 µm) obpyriform conidia (Markovskaja 2003).

With the addition of *C. sinensis*, *Cordana* is represented by 21 species (TABLE 1). All these species have integrated, polyblastic, terminal or intercalary, sympodial conidiogenous cells, except *C. bisbyi* (Timonin) Hern.-Restr. & al. and *C. terrestris* (Timonin) Hern.-Restr. & al., which bear discrete, polyblastic conidiogenous cells umbellately arranged at the apex of the conidiophores (Hernández-Restrepo & al. 2014). *Cordana* conidia are mostly smooth-walled and concolorous, but *C. indica* Subramon. & V.G. Rao and *C. verruculosa* Hern.-Restr. & al. produce echinulate or verruculose conidia. *Cordana inaequalis* S. Hughes, *C. mercadoana* Hern.-Restr. & al.,

Table 1 Comparisons of conidia and substrates of *Cordana* species. The new species is in bold.

| Species | Conidia | | | | Habitat | Type locality |
|---|---|---|---|---|---|---|
| | Size (μm) | Shape | Septation | Color | | |
| *C. abramovii*[1] | 18–31 × 12.5–15.5 | Ovoid | 1 | Uniform | Fruitbodies of *aphyllophoralean basidiocarp* and wood | Georgia |
| *C. andinopatagonica*[2] | 17–19 × 10–13 | Obovoid to ellipsoid | 1 | Uniform | Leaf litter of *Nothofagus dombeyi* | Argentina |
| *C. aquatica*[3] | 8–10 × 3–5 | Mostly oblong, sometimes ellipsoid | 1 | Uniform | Submerged decaying wood | China |
| *C. bisbyi*[4, 5,*] | 5–7.2 × 2.5–3.6 | Cylindrical–oval | 0 | Uniform | Soil | Honduras |
| *C. crassa*[6] | 20–24 × 13–15 | Ovoid | 1 | Uniform | Rotting wood of *Fagus sylvatica* | Hungary |
| *C. ellipsoidea*[7] | 8.7–11.5 × 4.3–4.7 | Ellipsoid to oblong | 1 | Uniform | Soil | Nepal |
| *C. inaequalis*[8] | 10–12.5 × 4–5 | Ellipsoidal to ovoid | 1 | Variable | Rotten wood | Canada |
| *C. indica*[9,**] | 11–20 × 4.5–7 | Oval to oblong | 1 | Uniform | Dead leaves of *Casearia* | India |
| *C. lignicola*[3] | 9–11 × 3.5–4.5 | Mostly oblong, sometimes ellipsoid, | 0–2 | Uniform | Submerged decaying wood | China |
| *C. lithuanica*[10] | 13–17.5 × 8–10 | Obpyriform | 1 | Uniform | Decaying wood of *Fraxinus excelsior* | Lithuania |
| *C. lushanensis*[11] | 5.5–8 × 2.5–4 | Ellipsoidal to obovoid | 0 | Uniform | Dead branches | China |

| Species | Conidia | | | | Habitat | Type locality |
|---|---|---|---|---|---|---|
| | Size (µm) | Shape | Septation | Color | | |
| *C. meilingensis*[11] | 10–13 × 5.5–7 | Oblong or cylindrical | 1 | Uniform | Dead branches | China |
| *C. mercadoana*[4] | 6–10 × 3–4 | Oblong, obovoid or cylindrical | 0–1 | Variable | Dead twig | Spain |
| *C. parasitica*[12] | 6–10 × 3–5 | Oblong | 1 | Variable | Living leaves of *Aster trinervi* var. *odusti* | Japan |
| *C. pauciseptata*[13] | 8–12 × 5–7 | Broad ellipsoid to ovoid | 1 | Uniform | Rotten wood of frondose tree | Germany |
| *C. semaniae*[14] | 21–27 × 9–15 | Obovoid | 0 | Uniform | Bark of rotten *Quercus* | Russia |
| ***C. sinensis*** | **13–20 × 5–7.5** | **Ovoid to obclavate** | **1–2** | **Uniform** | **Dead branches** | **China** |
| *C. solitaria*[15] | 6.5–9.5 × 4.5–6.5 | Obovoidal | 0 | Uniform | Rotten wood | India |
| *C. terrestris*[4,16,*] | 6–11 × 3–4 | Oblong to ovate | 1 | Uniform | Soil | Canada |
| *C. uniseptata*[17] | 13.5–23 × 8.5–11.5 | Mostly broad ellipsoid | 1 | Variable | *Phyllostachys bambusoides* submerged in forest stream | China |
| *C. verruculosa*[4,**] | 3–5.5 × 2–3.5 | Ellipsoidal to obovoid | 0 | Uniform | Dead wood | Spain |

*All conidiogenous cells are integrated polyblastic, except for *C. bisbyi* and *C. terrestris*, which are discrete polyblastic.

**All conidia are smooth, except for *C. indica*, which is echinulate and *C. verruculosa*, which is verruculose.

Data from: [1]Seman & Davydkina 1983; [2]Gamundí & al. 1979; [3]Luo & al. 2019; [4]Hernández-Restrepo & al. 2014; [5]Timonin 1961; [6]Tóth 1975; [7]de Hoog 1973; [8]Hughes 1983; [9] Subramoniam & Rao 1976; [10]Markovskaja 2003; [11]Ai & al. 2019; [12]Togashi & Onuma 1934; [13]Preuss 1851; [14]Davydkina & Mel'nik 1989; [15]Rao & de Hoog 1986; [16]Timonin 1940; [17]Cai & al. 2004.

*C. parasitica* Togashi & Onuma, and *C. uniseptata* L. Cai & al. produce versicolored conidia. Only five species produce nonseptate conidia: *C. bisbyi*, *C. lushanensis* C.C. Ai & al., *C. semaniae* Davydkina & al., *C. solitaria* V. Rao & de Hoog, and *C. verruculosa*, while most form 1-septate conidia, although in *C. lignicola* Z.L. Luo & al. and *C. sinensis*, 2-septate conidia are occasionally present. All 21 species except *C. parasitica* are usually found on rotten wood or dead branches, or in soil.

### Key to *Cordana* accepted species

1. Conidiogenous cells discrete, polyblastic, umbellately arranged over the swollen apex of the conidiophores ..... 2
1. Conidiogenous cells integrated, polyblastic, intercalary or terminal on the conidiophores ..... 3
2. Conidia 0-septate, cylindrical-oval, 5–7.2 × 2.5–3.6 µm ..... *C. bisbyi*
2. Conidia 1-septate, oblong to ovate, 6–11 × 3–4 µm ..... *C. terrestris*
3. Conidia nonseptate ..... 4
3. Conidia septate ..... 7
4. Conidia verruculose, 3–5.5 × 2–3.5 µm ..... *C. verruculosa*
4. Conidia smooth, ≥5.5 µm long ..... 5
5. Conidia obovoid, guttulate, 21–27 × 9–15 µm ..... *C. semaniae*
5. Conidia ≤9.5 × 6.5 µm ..... 6
6. Conidia ellipsoidal to obovoid, 5.5–8 × 2.5–4 µm ..... *C. lushanensis*
6. Conidia obovoidal, 6.5–9.5 × 4.5–6.5 µm ..... *C. solitaria*
7. Conidia oval to oblong, echinulate, 11–20 × 4.5–7 µm ..... *C. indica*
7. Conidia smooth, variably sized ..... 8
8. Conidia versicolorous ..... 9
8. Conidia concolorous ..... 12
9. Conidia ellipsoidal or ovoid ..... 10
9. Conidia oblong, obovoid, or cylindrical ..... 11
10. Conidia mostly broadly ellipsoidal, 13.5–23 × 8.5–11.5 µm ..... *C. uniseptata*
10. Conidia narrowly ellipsoidal to ovoid, 10–12.5 × 4–5 µm ..... *C. inaequalis*
11. Conidia oblong, only 1-septate, 6–10 × 3–5 µm ..... *C. parasitica*
11. Conidia oblong, obovoid or cylindrical, 0–1-septate, 6–10 × 3–4 µm ..... *C. mercadoana*
12. Conidia 0–2 or 1–2-septate ..... 13
12. Conidia only 1-septate ..... 14
13. Conidia mostly oblong, sometimes ellipsoid, 0–2-septate, 9–11 × 3.5–4.5 µm ..... *C. lignicola*
13. Conidia ovoid to obclavate, 1–2-septate, 13–20 × 5–7.5 µm ..... *C. sinensis*

14. Conidia of both cells more or less equally wide ..... 15
14. Conidia of both cells unequally wide ..... 18
15. Conidia ovoid, with a pigmented septal pore, 18–31 × 12.5–15.5 µm ..... *C. abramovii*
15. Conidia oblong, cylindrical, ellipsoid to oblong or ellipsoid to ovoid, lacking pigmented septal pore, ≤13 × 7 µm ..... 16
16. Conidia ellipsoid to oblong, 8.7–11.5 × 4.3–4.7 µm ..... *C. ellipsoidea*
16. Conidia ≥5 µm wide ..... 17
17. Conidia oblong or cylindrical, 10–13 × 5.5–7 µm ..... *C. meilingensis*
17. Conidia broadly ellipsoid to ovoid, 8–12 × 5–7 µm ..... *C. pauciseptata*
18. Conidia ovoid, 20–24 × 13–15 µm ..... *C. crassa*
18. Conidia obpyriform, obovoid, ellipsoid or oblong, ≤19 × 13 µm ..... 19
19. Conidia mostly oblong, sometimes ellipsoid, 8–10 × 3–5 µm ..... *C. aquatica*
19. Conidia ≥13 × 8 µm ..... 20
20. Conidia obovoid to ellipsoid, 17–19 × 10–13 µm ..... *C. andinopatagonica*
20. Conidia obpyriform, 13–17.5 × 8–10 µm ..... *C. lithuanica*

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# *Pezicula endophytica* sp. nov., endophytic in *Dendrobium* in Thailand

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Abstract—A new species, *Pezicula endophytica,* was isolated from roots and stems of two *Dendrobium* species in northern Thailand. Evidence to support the new species is based on morphology and phylogenetic analysis of the combined ITS, LSU, and RPB2 DNA sequence dataset. *Pezicula endophytica,* which constituted a clade independent from other *Pezicula* species, has 4% distinct base pair differences in all genes. *Pezicula endophytica* has larger macroconidia and longer conidiophores compared with phylogenetically neighboring species. This is the first report of an endophytic *Pezicula* species from *Dendrobium* in Thailand.

Key words— ascomycetes, *Cryptosporiopsis*, *Dermateaceae*, multi-loci, *Orchidaceae*

## Introduction

Fungal endophytes in orchids may be symbionts that help plant growth and are possible alternative sources for the bioactive compounds produced

in plants (Bungtongdee & al. 2019, Ma & al. 2015, Vujanovic & al. 2000). They also play a role as mycorrhizae in orchids and transfer water and nutrition to the host (Bungtongdee & al. 2019, Ma & al. 2015, Smith & Read 2010). *Pezicula* Tul. & C. Tul. (*Dermateaceae*; anamorph: *Cryptosporiopsis* Bubák & Kabát) is apothecial (Ekanayaka & al. 2017, 2019). The name *Pezicula* has been proposed as the accepted name because *Cryptosporiopsis* species are the asexual morphs of both *Pezicula* and *Neofabraea* H.S. Jacks. (Chen & al. 2016; Johnston & al. 2014; Verkley 1999; Verkley & al. 2003; Wijayawardene & al. 2017a,b). *Pezicula* is characterized by brightly colored, short-stalked apothecia with circular discs that are initially concave and surrounded by slightly elevated margins but later become convex. They occur on the bark of woody plants and are involved in wood degradation (Kowalski 1996). Asci are inoperculate and mostly clavate or cylindric-clavate, ascospores vary in shape from broadly ellipsoid to elongate-ellipsoid to allantoid or fusoid (Ekanayaka & al. 2016, Ooki & al. 2003, Verkley 1999, Verkley & al. 2003). The asexual morph of *Pezicula* species produces sporodochial conidiomata, with macro- and microconidia formed on different conidiophores (Ekanayaka & al. 2016, Verkley 1999). *Pezicula* species are recorded as endophytes, plant pathogens, and saprobes from a wide range of hosts (Chen & al. 2016, Wijayawardene & al. 2017a). They have been isolated as endophytes from *Dendrobium nobile* Lindl. and *Holcoglossum wangii* Christenson (*Orchidaceae*) in China (Chen & al. 2011, Tan & al. 2012).

*Dendrobium* Sw. is one of the largest genera in *Orchidaceae* (https://www.kew.org/), and many species are important medicinal and ornamental plants (Ma & al. 2015). However, few reports concern endophytic *Pezicula* species in *Dendrobium* (Chen & al. 2011, Tan & al. 2012).

In this study, we isolated four endophytic fungal strains from two different, undetermined *Dendrobium* species sampled in northern Thailand. The strains were identified as representing a new species, *Pezicula endophytica,* based on morphological and phylogenetic analyses. This is the first report of an endophytic *Pezicula* in *Dendrobium* in Thailand.

## Materials & methods

### Sample collection

Healthy roots and stems of two different but undetermined *Dendrobium* species (sp.1 and sp.2) were collected from outside the temple of Wat Phra That Doi Tung (Temple of Doi Tung Pagoda), Mae Fah Luang district, Chiang Rai Province,

Thailand. Fresh material was kept fresh by placing into Zip-lock bags or tubes containing silica gel on ice.

#### Fungal isolation & cultivation

The material was processed within 48 hours. Surface sterilization followed Nontachaiyapoom & al. (2010) with minor modifications. Healthy roots and stems were washed in tap water and then immersed for 5 minutes in a solution containing 3% (v/v) $H_2O_2$ and 70% (v/v) ethanol, and then rinsed with three changes of sterile distilled water. The sterilized materials were cut into 2 $mm^2$ pieces and placed on potato dextrose agar (PDA, Difco, BBL/USA' #213400) containing 50 µg/ml oxytetracycline, 50 µg/ml penicillin, and 50 µg/ml streptomycin (Otero & al. 2002). The surface sterilization method was checked, as described by Petrini (1991). Petri dishes were incubated at 28 °C under natural light and observed daily; mycelia on the edge of developing fungal colonies were transferred to fresh PDA to obtain pure cultures. The pure cultures were deposited in Mae Fah Luang University Culture Collection, Chiang Rai, Thailand (MFLUCC). Dry cultures of the new species were deposited in the herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU).

#### Morphological analysis

Pure cultures were cultivated on both PDA and water agar (WA) media in a dark cabinet at room temperature (28 °C) and observed every week. Sterilized toothpicks were placed on the WA surface to encourage fungal sporulation. The growth rate was evaluated after mycelia had nearly covered the whole agar surface. Any observable structures were examined using a Zeiss SteREO Discovery.V8 stereomicroscope. The colonies were examined for morphological characteristics and structures were removed and mounted in water. The mounted slides were observed using a Nikon EOS 700D compound microscope. All observed structures were measured with Image Frame Work v.0.9.7 and the photo plates were assembled using Photoshop CS 6.0.

#### DNA extraction & amplification

Genomic DNA was extracted from mycelium of endophytic fungi using an EZgene Fungal gDNA Kit following the manufacturer's instructions. The selected genes for amplification were partial nuclear rDNA internal transcribed spacer (ITS), partial large subunit (LSU), and partial RNA polymerase II large subunit 2 (RPB2). Each 25 µl amplification reaction contained 12.5 µl of 2*Bench TopTM Taq Master Mix (0.05 units/µl Taq DNA polymerase, 0.4 mM dNTPs and 4 mM $MgCl_2$); 2 µl forward and reverse primers; 1 µl of DNA template and 9.5 µl of threefold-distilled water. The primers and PCR protocol followed Chen & al. (2016). PCR products were visualized on 1% agarose gel stained with Realtimes Biotech Goldview and then sent to Sangon Biotech for purification and sequencing. All DNA sequences from this study were submitted to GenBank.

Table. 1. Fungal strains and sequences of *Pezicula* and related taxa sourced from the present study, BLAST searches in GenBank, or Chen & al. (2016).

| Species | Strain | GenBank accession No. | | |
|---|---|---|---|---|
| | | ITS | LSU | RPB2 |
| *Neofabraea.inaequalis* | **CBS 326.75** | KR859081 | KR858872 | KR859321 |
| *N. krawtzewii* | CBS 102867 | KR859084 | KR858875 | KR859324 |
| *N. malicorticis* | CBS 102863 | KR859085 | KR858876 | KR859325 |
| | **CBS 122030** | KR859086 | KR858877 | KR859326 |
| *Parafabraea caliginosa* | **CBS 124806** | KR859090 | KR858881 | KR859330 |
| *Pa. eucalypti* | **CBS 124810** | KR859091 | KR858882 | KR859331 |
| *Pezicula acericola* | CBS 239.97 | KR859093 | KR858884 | KF376214 |
| | CBS 245.97 | KR859098 | KR858889 | KF376213 |
| *P. aurantiaca* | CBS 201.46 | KR859102 | KR858893 | KF376210 |
| *P. brunnea* | **CBS 120291** | KR859103 | KR858894 | — |
| *P. californiae* | **CBS 124805** | KR859104 | KR858895 | KR859332 |
| *P. carpinea* | **CBS 923.96** | KR859108 | KR858899 | KF376158 |
| | CBS 921.96 | KR859107 | KR858898 | KF376159 |
| *P. chiangraiensis* | **MFLUCC 15-0170** | KU310621 | KU310622 | KU310623 |
| *P. cinnamomea* | CBS 626.96 | KR859152 | KR858944 | KF376162 |
| | CBS 239.96 | KR859124 | KR858915 | KF376165 |
| *P. cornina* | CBS 285.39 | KR859163 | KR858955 | KR859333 |
| *P. corticola* | CBS 259.31 | KR859164 | KR858956 | — |
| | CBS 260.31 | KR859165 | KR858957 | — |
| *P. corylina* | CBS 140.22 | KR859166 | KR858958 | KR859334 |
| | CBS 249.97 | KR859168 | KR858960 | KF376161 |
| *P. diversispora* | **CBS 185.50** | KR859170 | KR858962 | — |
| | CBS 282.47 | KR859171 | KR858963 | — |
| ***P. endophytica*** | **MFLUCC 14-0116** | MN908669 | MN908642 | MT371880 |
| | MFLUCC 14-0118 | MN908666 | MN908640 | — |
| | MFLUCC 14-0140 | MN908667 | MN908641 | — |
| | MFLUCC 14-0144 | MN908668 | — | — |
| *P. ericae* | **CBS 120290** | KR859173 | KR858965 | — |
| | CBS 120292 | KR859174 | KR858966 | — |
| *P. eucrita* | CBS 259.97 | KR859179 | KR858971 | KF376205 |
| | CBS 656.96 | KR859185 | KR858977 | KF376208 |
| *P. fagacearum* | **CBS 112400** | KR859201 | KR858993 | KR859335 |
| | CBS 112402 | KR859203 | KR858995 | KR859336 |
| *P. frangulae* subsp. *frangulae* | CBS 100244 | KR859204 | KR858996 | KF376211 |
| | CBS 778.96 | KR859209 | KR859001 | KF376212 |
| *P. heterochroma* | **CBS 199.46** | KR859210 | KR859002 | — |
| *P. melanigena* | **CBS 898.97** | KR859211 | KR859003 | — |
| *P. microspora* | **CBS 124641** | KR859212 | KR859004 | KR859337 |
| *P. neocinnamomea* | **CBS 100248** | KR859213 | KR859005 | KF376209 |
| *P. neoheterochroma* | **CBS 127388** | KR859221 | KR859013 | KR859338 |

| Species | Strain | GenBank accession No. | | |
|---|---|---|---|---|
| | | ITS | LSU | RPB2 |
| *P. neosporulosa* | **CBS 101.96** | KR859223 | KR859015 | KF376193 |
| | CBS 102.96 | KR859224 | KR859016 | KF376181 |
| *P. ocellata* | CBS 268.39 | KR859232 | KR859024 | KR859339 |
| | CBS 949.97 | KR859233 | KR859025 | KF376215 |
| *P. pruinosa* | CBS 292.39 | KR859234 | KR859026 | — |
| *P. pseudocinnamomea* | **CBS 101000** | KR859235 | KR859027 | KR859340 |
| *P. querciphila* | **CBS 134525** | JX144750 | — | — |
| *P. radicicola* | **CBS 640.94** | KR859236 | KR859028 | — |
| | CBS 681.83 | KR859237 | KR859029 | — |
| *P. rubi* | CBS 253.97 | KR859250 | KR859042 | KF376204 |
| | CBS 593.96 | KR859253 | KR859045 | KF376203 |
| *P. sporulosa* | **CBS 224.96** | KR859261 | KR859053 | KF376201 |
| | CBS 225.96 | KR859262 | KR859054 | KF376202 |
| *Phlyctema vagabunda* | CBS 109875 | KR859275 | KR859069 | KR859346 |
| | **CBS 304. 62** | KR859276 | KR859070 | KR859347 |
| *Rhizodermea veluwensis* | **CBS 110605** | KR859282 | KR859076 | KR859353 |
| | CBS 110615 | KR859283 | KR859077 | KR859354 |

*Ex-type, ex-epitype or other type strains are in **bold**

### Sequence analysis

The sequencing results were assembled using DNASTAR SeqMan v.7.1.0. The assembled sequences were subjected to nucleotide BLAST searches in GenBank (www.ncbi.nlm.nih.gov/BLAST) for establishing highly similar strains (Altschul & al. 1997). Supplemental strains of *Pezicula* and related genera were selected from Chen & al. 2016, especially ex-type and ex-epitype sequences where available. Sequence alignment was carried out by MAFFT v.7.0 (https://mafft.cbrc.jp/alignment/server/). Misaligned sequences were manually edited using AliView v.1.26 (Larsson 2014). The three loci were combined into a concatenated dataset with SequenceMatrixa 1.7.8 (Vaidya & al. 2011).

The best fitting nucleotide substitution models for each gene were estimated by IQ-TREE (Nguyen & al. 2014) with ultrafast bootstrap (Hoang & al. 2017). Gaps were regarded as missing data. Both maximum likelihood (ML) and Bayesian (BI) trees were constructed on The CIPRES Science Gateway V. 3.3 (http://www.phylo.org/index.php/). The ML tree was constructed using RAxML-HPC2 on XSEDE. Maximum likelihood bootstrap values (MLSB) were calculated with 1000 iterations. The generalized time-reversible (GTR) was set with gamma distribution. The BI tree was implemented by MrBayes on XSEDE (3.2.6). Two sets of four simultaneous independent chains of Markov chains Monte Carlo (MCMC) simulations were run for 5,000,000 generations; 25% of trees were discarded and the remaining trees were used to calculate the Bayesian posterior probabilities (PP). Convergence was assumed

when the standard deviation of split sequences was less than 0.01. The generated trees were submitted to TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S25576). The resulting trees were viewed with Figtree v.1.4.0. The layout was made with Adobe Illustrator CS 6.

## Results

### Fungal isolation & sporulation

Four endophytic *Pezicula* strains were isolated from healthy roots and stems of *Dendrobium* species. Isolate MFLUCC 14-0116 sporulated on PDA after one month and on WA with toothpicks after two months. The conidia, conidiomata, conidiophores and mycelia were recorded from cultures.

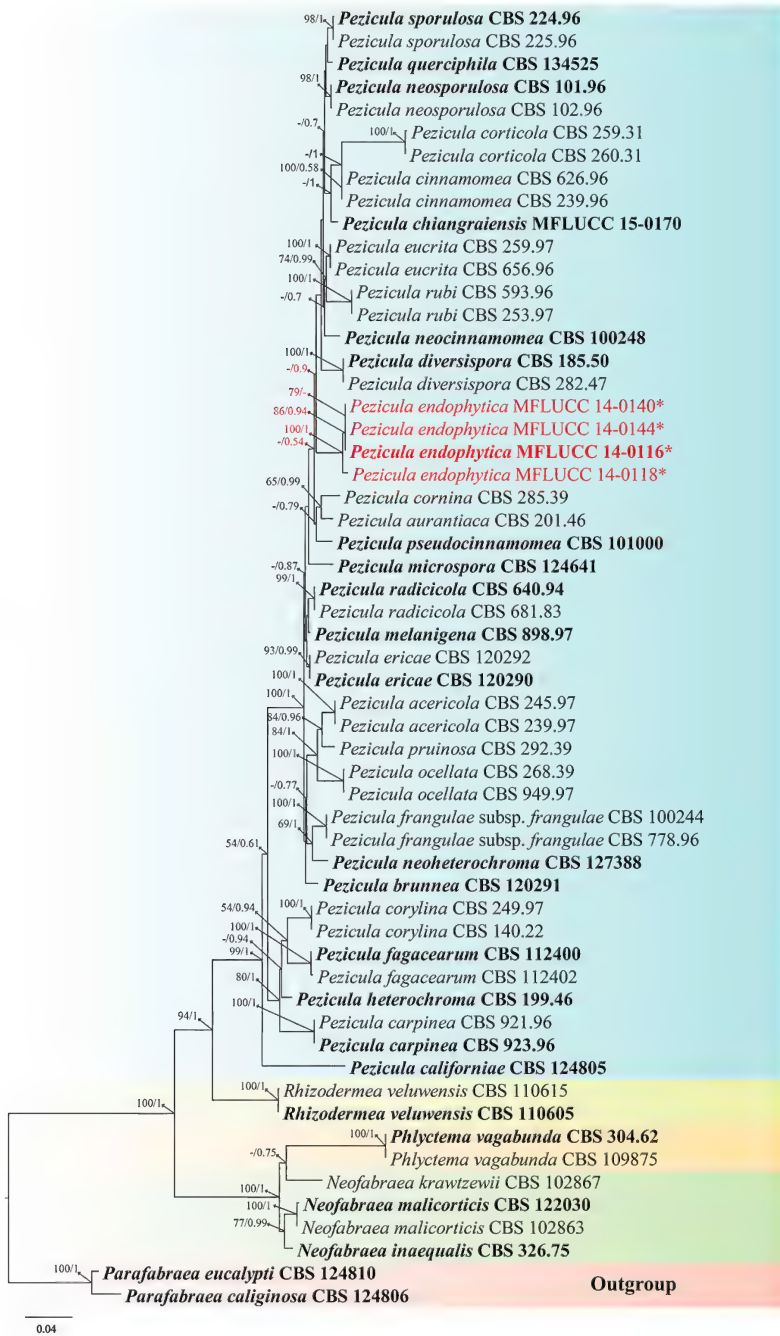
### Phylogenetic results

Sequences of the isolated *Pezicula* and reference sequences of related taxa are listed in Table 1. Combined data for the phylogenetic tree comprised 57 sequences containing 2478 sites (including 582 ITS sites, 843 LSU sites, and 1053 RPB2 sites). The selected nucleotide substitutional model for ITS and RPB2 was a symmetrical model (SYM) with gamma distribution, and for LSU was Kimura's two-parameter substitution model with invgamma distribution.

The phylogenetic tree (Fig. 1) was rooted with two selected outgroup species *Parafabraea caliginosa* (CBS 124806) and *P. eucalypti* (CBS 124810) with robust bootstrap support (ML = 100%, PP = 1). In the phylogenetic tree (RAxML), *Pezicula endophytica* strains clustered and formed an independent clade. Strains MFLUCC 14-0140, MFLUCC 14-0144 and MFLUCC 14-0116 clustered with MFLUCC 14-0118 (ML = 100%, PP = 1). *Pezicula aurantiaca*, *P. cornina* and *P. pseudocinnamomea* formed a clade close to *P. endophytica*. The neighboring genera *Neofabraea* H.S. Jacks., *Phlyctema* Desm. and *Rhizodermea* Verkley & Zijlstra were well separated from *Pezicula* (ML = 94%, PP = 1).

*Pezicula endophytica* (MFLUCC 14-0116, ex-type) differs from *Pezicula diversispora* (CBS 185.50, ex-type) in 4% of base pairs [2.9% in ITS (17/582bp), 1.1% in LSU (9/843bp)] and from *Pezicula cornina* (CBS 285.39) in 8.6% of base pairs [3.3% in ITS (19/582bp); 1.5% in LSU (13/843bp); 3.8% in RPB2 (40/1053bp)].

Fig. 1. The consensus phylogram resulting from a RAxML analysis of the combined three loci (ITS-LSU-RPB2) of *Pezicula* and related taxa. *Parafabraea caliginosa* and *P. eucalypti* were selected as outgroup taxa. Isolates generated in this study are labelled with *. Holotype and ex-type isolates are in bold. Support values from maximum likelihood (MLBS) and Bayesian posterior probabilities (PP) are labelled at the end of nodes (50% majority rule). Dashes indicate values ≤50%. Scale bar represents 0.04 substitutions per site.


98/1
Pezicula sporulosa CBS 224.96
Pezicula sporulosa CBS 225.96
Pezicula querciphila CBS 134525
98/1
Pezicula neosporulosa CBS 101.96
Pezicula neosporulosa CBS 102.96
-/0.7
100/1
Pezicula corticola CBS 259.31
Pezicula corticola CBS 260.31
-/1
100/0.58
-/1
Pezicula cinnamomea CBS 626.96
Pezicula cinnamomea CBS 239.96
Pezicula chiangraiensis MFLUCC 15-0170
100/1
74/0.99
Pezicula eucrita CBS 259.97
Pezicula eucrita CBS 656.96
100/1
-/0.7
Pezicula rubi CBS 593.96
Pezicula rubi CBS 253.97
Pezicula neocinnamomea CBS 100248
100/1
-/0.9
Pezicula diversispora CBS 185.50
Pezicula diversispora CBS 282.47
79/-
86/0.94
100/1
-/0.54
Pezicula endophytica MFLUCC 14-0140*
Pezicula endophytica MFLUCC 14-0144*
Pezicula endophytica MFLUCC 14-0116*
Pezicula endophytica MFLUCC 14-0118*
65/0.99
-/0.79
Pezicula cornina CBS 285.39
Pezicula aurantiaca CBS 201.46
Pezicula pseudocinnamomea CBS 101000
-/0.87
Pezicula microspora CBS 124641
99/1
Pezicula radicicola CBS 640.94
Pezicula radicicola CBS 681.83
Pezicula melanigena CBS 898.97
93/0.99
Pezicula ericae CBS 120292
100/1
Pezicula ericae CBS 120290
100/1
Pezicula acericola CBS 245.97
84/0.96
84/1
Pezicula acericola CBS 239.97
Pezicula pruinosa CBS 292.39
100/1
Pezicula ocellata CBS 268.39
-/0.77
100/1
Pezicula ocellata CBS 949.97
69/1
Pezicula frangulae subsp. frangulae CBS 100244
Pezicula frangulae subsp. frangulae CBS 778.96
54/0.61
Pezicula neoheterochroma CBS 127388
Pezicula brunnea CBS 120291
54/0.94
100/1
Pezicula corylina CBS 249.97
100/1
Pezicula corylina CBS 140.22
-/0.94
99/1
Pezicula fagacearum CBS 112400
80/1
Pezicula fagacearum CBS 112402
100/1
Pezicula heterochroma CBS 199.46
94/1
Pezicula carpinea CBS 921.96
Pezicula carpinea CBS 923.96
Pezicula californiae CBS 124805
100/1
Rhizodermea veluwensis CBS 110615
100/1
Rhizodermea veluwensis CBS 110605
100/1
Phlyctema vagabunda CBS 304.62
-/0.75
Phlyctema vagabunda CBS 109875
Neofabraea krawtzewii CBS 102867
100/1
Neofabraea malicorticis CBS 122030
100/1
77/0.99
Neofabraea malicorticis CBS 102863
Neofabraea inaequalis CBS 326.75
100/1
Parafabraea eucalypti CBS 124810
Parafabraea caliginosa CBS 124806
Outgroup
0.04

## Taxonomy

***Pezicula endophytica*** X.Y. Ma, K.D. Hyde & J.C. Kang, sp. nov. FIG. 2

IF 558634

Differs from *Pezicula diversispora* by its longer conidiophores and narrower macroconidia.

TYPE—Thailand, Chiang Rai Province, Mae Fah Luang district, outside Temple of Doi Tung Pagoda, endophytic in the roots of *Dendrobium* sp. 1, 19 December 2013, Nontachaiyapoom S, Aewsakul N & Ma XY (**holotype**, MFLU 17-2789; ex-type living culture, MFLUCC 14-0116; **isotype**, MFLU 20-0442; ex-type living culture, MFLUCC 14-0118.

ETYMOLOGY—in reference to its endophytic habit.

SEXUAL MORPH undetermined. ASEXUAL MORPH observed on both PDA and (with toothpicks) WA.

COLONIES on WA superficial, white with light brownish grey mycelia, sparse, irregular edge, fluffy, reverse light brown, growth rate 0.8 mm/day. MYCELIUM hyaline to light brown, smooth, 2.5–4.4 µm diam. CONIDIOMATA on toothpicks on WA aggregated or scattered, with aerial and fluffy hyphae, yellow-grey, globose.

COLONIES on PDA medium superficial, white and brown in the center, lobate edge, dense, fluffy, concentric ring close to the edge, reverse brown, growth rate: 1.2 mm/day. CONIDIOMATA erumpent on surface, aggregated or scattered, with irregular sporodochia, with brown to milky white conidial masses, globose to irregular. MACROCONIDIOPHORES (16–)22–30(–40) × (2–)3–4(–5.5) µm ($x$ = 26 × 3.5 µm, n=5), arising from the inner wall layers of conidiomata, hyaline, cylindrical, straight to flexuous, septate, unbranched or branched, smooth-walled. MACROCONIDIOGENOUS CELLS hyaline, enteroblastic, phialidic, determinate, proliferating percurrently, sometimes with a minute collarette, cylindrical, integrated, determinate, smooth-walled. MACROCONIDIA (30–)31–43(–44) × (5–)7–9 (–10) µm ($x$ = 37 × 8 µm, n=16), L/W=4.4, hyaline, elongated ellipsoid, rounded at the apex, narrow and slightly truncate at the base, sometimes attenuated and with a protruding scar at the base, septate when mature, straight, smooth-walled, with guttules.

ADDITIONAL SPECIMENS EXAMINED—**THAILAND, CHIANG RAI PROVINCE, Mae Fah Luang district**, outside Temple of Doi Tung Pagoda, endophytic in roots and stems of *Dendrobium* sp. 2, 19 December 2013, Nontachaiyapoom S, Aewsakul N & Ma XY. (MFLUCC 14-0140, GenBank MN908667, MN908641; MFLUCC 14-0144, GenBank MN908668).

COMMENTS—*Pezicula endophytica* formed an independent clade differing from other species in the phylogenetic tree, and is adjacent to *P. aurantiaca*,

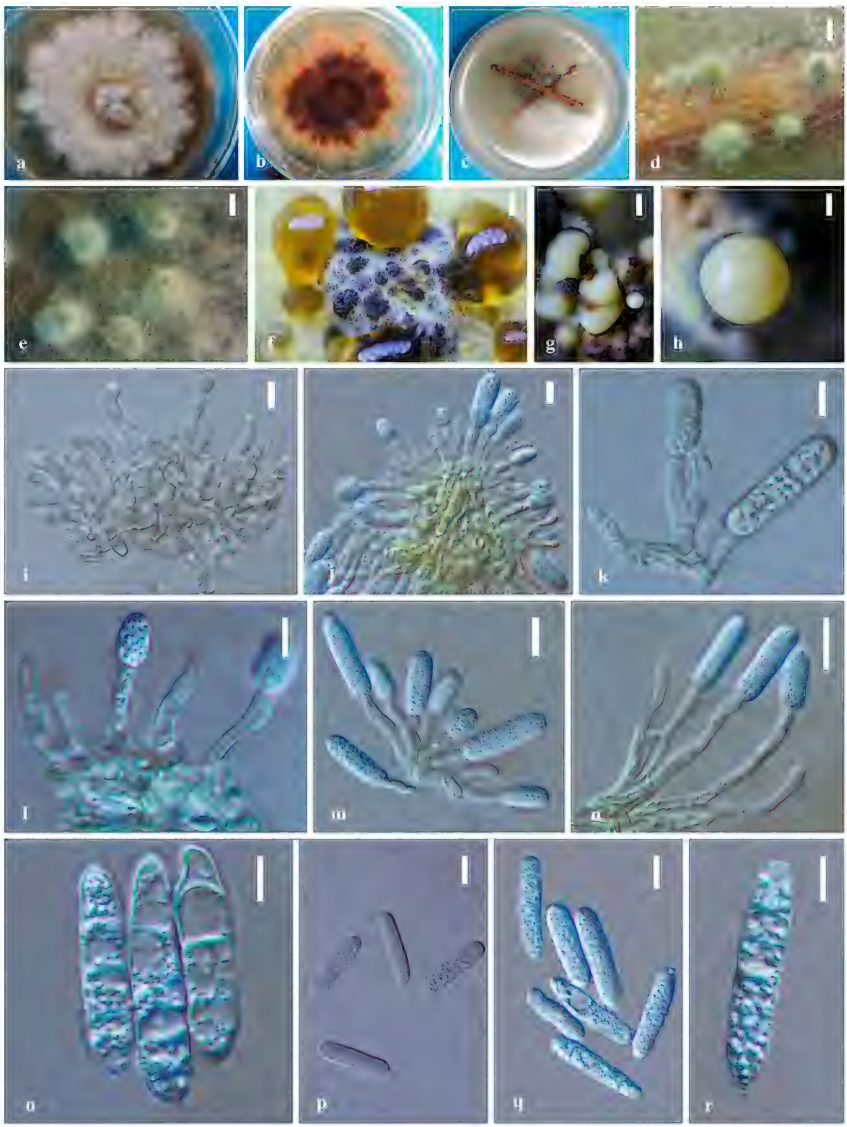


Fig. 2. *Pezicula endophytica* (ex-holotype MFLUCC 14-0116). a, b. Colony on PDA (a. top; b. reverse). c. Colony on WA with toothpicks. d, e. Conidiomata on toothpicks on WA. f–h. Conidiomata with conidial masses on PDA. i–n. Macroconidiophores, macroconidiogenous cells and macroconidia. o–r. Macroconidia. Note: i–r from PDA. Scale bars: d–h = 500 µm; i–n, p, q = 10 µm; o, r = 5 µm.

*P. cornina*, *P. diversispora,* and *P. pseudocinnamomea*. *Pezicula endophytica* is distinguished from those species by its longer macroconidiophores and narrower macroconidia with septa (*P. aurantiaca* and *P. cornina* are aseptate; Chen & al. 2016, Robak 1950, Verkley 1999). *Pezicula endophytica* (ex-type MFLUCC 14-0116) has >4% different gene sequences than *P. diversispora* (ex-type CBS 185.50). Compared with their cultures on MEA, *Pezicula endophytica* produces no diffusing pigment on WA (Verkley 1999). *Pezicula aurantiaca*, *P. cornina*, *P. diversispora,* and *P. pseudocinnamomea* were isolated from dead branches, bark, and twigs of dicotyledonous woody plants from European countries (Chen & al. 2016, Robak 1950, Verkley 1999), whereas *P. endophytica* is an endophytic fungus from monocotyledonous *Dendrobium* orchids.

## Discussion

The taxonomy and identification of *Pezicula* is complicated because many species have been introduced based on single-morph taxa, some lack molecular data, and their morphology may vary depending on the growing medium (Verkley 1999, Verkley & al. 2003). Of the 136 species listed in *Pezicula* (http://www.speciesfungorum.org), only 29 (including *P. endophytica*) have DNA sequence data available in the 2021 NCBI database (Yuan & al. 2015, Ekanayaka & al. 2016; http://www.ncbi.nlm.nih.gov/). A revision of the genus based on comprehensive morphological and phylogenetic data is necessary. The asexual morphs of most *Pezicula* species produce both macro- and microconidia on the natural substrata and in cultures (Verkley 1999). In this study, only macroconidia were observed in cultures. Macroconidia of *P. endophytica* resemble *P. diversispora,* which differs by its longer conidiophores, narrower macroconidia, and a 4% gene sequence difference. *Pezicula endophytica,* which only produced its asexual morph in cultures, differs from *P. carpinea* (the type species) by lacking conidia along the conidiophore sides and large gene sequence differences. Presumably *P. endophytica* microconidia will not be produced on WA after two sporulation months.

Chen & al. (2011) first reported an endophytic *Pezicula* sp. from *Dendrobium* orchids in China. *Pezicula endophytica* was the first *Pezicula* endophyte identified in Thailand. *Pezicula* species are frequently isolated endophytes in angiosperms, particularly in roots of *Abies*, *Calluna*, *Erica*, *Larix*, *Pleurothallis*, *Pseudorchis*, *Stelis,* and *Vaccinium* (Chen & al. 2016, Devi & Joshi 2014, Herrera & al. 2010, Kohout & al. 2013, Noble & al. 1991,

Schulz & al. 2002, Strobel & al. 1999, Talontsi & al. 2012, Verkley & al. 2003, Wang & al. 2014, Yuan & Verkley 2015, Zilla & al. 2013). They have also been found in peridermal bark of living branches of temperate trees such as *Abies*, *Acer*, *Alnus*, *Betula*, *Carpinus, Castanea, Fagus*, *Fraxinus*, *Picea*, *Pinus*, *Quercus,* and *Sequoia* (Espinosa-Garcia & Langenheim 1990, Kowalski & Kehr 1992, Matsumura & al. 2013, Petrini & Müller 1979, Schulz & al. 1995, Sieber 1988, Sieber & Dorworth 1994, Verkley 1999).

Although many *Pezicula* species are reported from temperate and boreal forests of the northern hemisphere, such as Canada, Germany, and the Netherlands (Kowalski & Kehr 1992, Chen & al. 2016, Verkley 1999), few geographic and climatic limitations have been observed. Saprobic and pathogenic *Pezicula* species have been recorded from temperate to tropical and Mediterranean areas (Bergero & al. 2003, Cheewangkoon & al. 2010, Old & al. 2002). *Pezicula eucalyptigena* was isolated from leaves of *Eucalyptus* sp. in the mediterranean Western Cape, South Africa, which has smaller conidia ((23–)24–27(–30) × (6–)7(–8) µm; Crous & al. 2019) than those of *P. endophytica*. Ekanayaka & al. (2016) described a sexual morph from Thailand, *P. chiangraiensis*, saprobic on the bark of decaying wood. *Pezicula ericae* was found as ericoid mycorrhizal fungi in the roots of velvetleaf blueberry (*Vaccinium myrtilloides*) seedlings in Canada, which could enhance development of the host plant (Mu 2020). For most endophytic *Pezicula* species, their roles remain unknown.

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# *Globoramichloridium delicatum* and *Heteroconium simile* spp. nov. from southern China

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Abstract — Two new anamorphic fungi, *Globoramichloridium delicatum* and *Heteroconium simile*, collected from dead branches of unidentified broadleaf trees in Jiangxi Province, China, are described and illustrated. *Globoramichloridium delicatum* is characterized by unicellular, subglobose to narrow subnapiform, yellowish-green conidia. *Heteroconium simile* is distinguished by its monoblastic, integrated, terminal, determinate conidiogenous cells that produce acrogenous, blastocatenate, dimorphic, 0–1-septate, smooth conidia.

Key words—*Antennulariellaceae*, *Dissoconiaceae*, *Dothideomycetes,* hyphomycetes, taxonomy

## Introduction

Saprobic dematiaceous hyphomycetes are highly diverse on rotting woody plant materials (Xia & al. 2014, 2016). As essential components in forest ecosystems, they play important roles in nutrient recycling, but their geographical distribution and alpha taxonomy are poorly known. In order to enrich the inventory of saprobic dematiaceous hyphomycetes in China, several

studies on fungi occurring on dead branches have been conducted during the last 15 years (Zhang & Shi 2005; Ma & Zhang 2007, 2015; Ma & al. 2008, 2014, 2016; Zhang & al. 2009, 2014; Xia & al. 2012, 2015; Xu & al. 2017, 2019; Qiu & al. 2020).

Jinggangshan Mountain is a small range in southeast Jiangxi, China. It lies in the center part of Luoxiao Mountains, which border both Hunan and Jiangxi provinces. Its scenic area covers c. 260 km$^2$ and exhibits complex geographical and topographical conditions with an 81.2% forest coverage rate and a 14.2 °C annual average temperature. It is considered one of the world's most abundant biological areas within this latitudinal zone. A field trip was made to Jinggangshan Mountain to collect saprobic hyphomycetes in November 2014. Two new species with morphologic features typical of *Globoramichloridium* Y. Marín & Crous (Marin-Felix & al. 2019; *Dissoconiaceae*) and *Heteroconium* Petr. (Petrak 1949; *Antennulariellaceae*) were collected on dead branches. The two new fungal species are described and illustrated below.

## Materials & methods

Dead branches were collected from humid environments and watersides in the forest ecosystems of Jinggangshan Mountain and placed in Ziploc™ plastic bags for transport to the laboratory, where they were processed, and examined as described by Ma & al. (2011). Microphotographs were prepared using a Nikon Eclipse E200 microscope equipped with a SmartV550Dc digital camera, with a 100× (oil immersion) objective at the same background and scale. Conidia were measured at their widest point. The range between minimum and maximum values for microscopic measurements is given with outliers in parentheses. Adobe Photoshop 7.0 was used to assemble photographs into plates. The specimens are deposited in the Herbarium of Jiangxi Agricultural University, Plant Pathology, Nanchang, Jiangxi, China (HJAUP).

## Taxonomy

***Globoramichloridium delicatum*** L. Qiu, Jian Ma, R.F. Castañeda & X.G. Zhang, **sp. nov.** Fig. 1

MB 836851

Differs from *Globoramichloridium indicum* by its larger unicellular subglobose to narrow subnapiform yellowish green conidia.

Type: China, Jiangxi Province: Jinggangshan Mountain, Shuikou scenic spot, 26°33′03″N 114°07′55″E, on dead branches of an unidentified broadleaf tree, 6 Nov. 2014, J. Ma (**Holotype**, HJAUP M0217).

Etymology: Latin, *delicatum*, meaning "delicate", referring to the dainty conidia.

Colonies on the natural substratum, effuse, brown. Mycelium superficial and immersed composed of branched, septate, pale brown or brown,

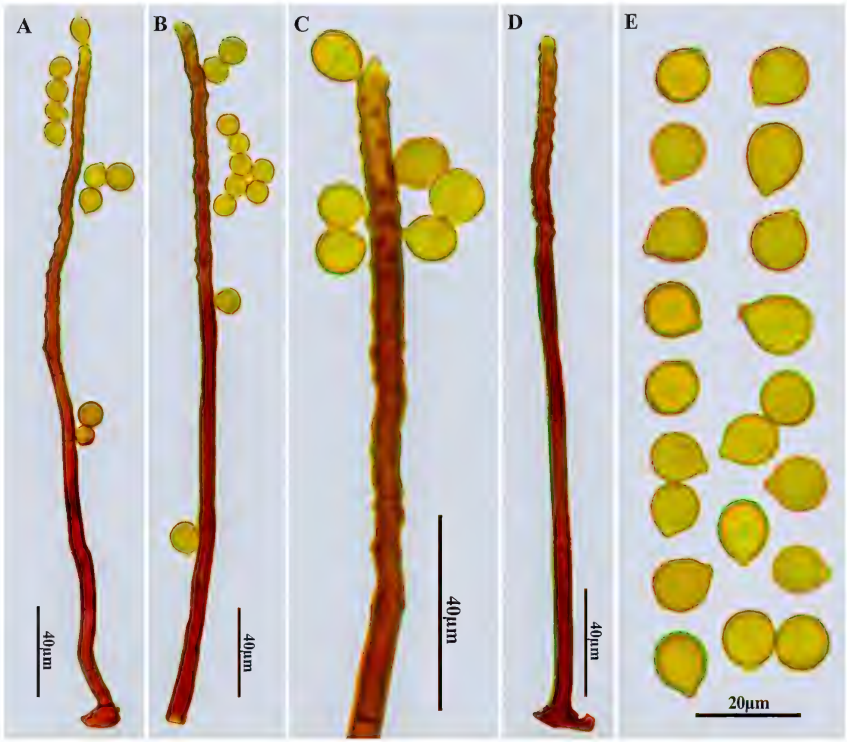


Fig. 1. *Globoramichloridium delicatum* (holotype, HJAUP M0217).
A–C. Conidiophores, conidiogenous cells, and conidia; D. Conidiophore; E. Conidia.

smooth-walled hyphae. Conidiophores macronematous, mononematous, unbranched, erect, straight or flexuous, cylindrical, 4–9-septate, smooth, brown to dark brown, paler towards the apex, ≤310 μm long, 6–8 μm wide. Conidiogenous cells polyblastic, integrated, terminal, sympodial extended, with crowded, prominent, thickened, slightly melanized scars. Conidia solitary, acropleurogenous, subglobose to narrow subnapiform, unicellular, yellowish green, verrucose or smooth, dry, 11–14 × 9.5–13 μm, truncate at the base, 1–2 μm wide, with a conspicuous 1 μm long hilum.

Comments –The monotypic genus *Globoramichloridium* was erected based on molecular phylogenetic analysis, with *Chloridium indicum* Subram. (Subramaniam 1955) [≡ *Globoramichloridium indicum* (Subram.) Y. Marín & Crous] as its type (Marin-Felix & al. 2019). *Globoramichloridium* is

characterized by broadly ellipsoidal to globose, (0–)1-septate, smooth-walled or coarsely verrucose conidia seceding schizolytically from polyblastic, integrated, terminal, sympodial, geniculate or nodose conidiogenous cells with prominent, thickened and darkened scars (Marin-Felix & al. 2019). These morphological characteristics differ from the similar genus *Ramichloridium* Stahel ex de Hoog (de Hoog & Hermanides-Nijhof 1977), which has conspicuous conidiogenous loci with crowded or scattered, unpigmented to faintly pigmented, or slightly prominent denticles, thin scars, and clavate or oblong to ellipsoid, or obovate to obconical, aseptate, smooth-walled to finely verrucose conidia (Arzanlou & al. 2007, Seifert & al. 2011).

*Globoramichloridium* was previously known only from the type species, *G. indicum* [≡ *Chloridium indicum*; ≡ *Veronaea indica* (Subram.) M.B. Ellis; ≡ *Ramichloridium indicum* (Subram.) de Hoog], which has been reported from Australia, Cuba, Hong Kong, Malaysia, and Brazil (Castañeda-Ruíz 1986, GBIF 2020). *Globoramichloridium delicatum,* the second species of the genus, differs from *G. indicum*, which has smaller [(5–)7–8(–10) × (4–)6–6.5(–9) µm], broadly ellipsoidal to globose, (0–)1-septate, subhyaline to pale brown conidia (Arzanlou & al. 2007).

***Heteroconium simile*** L. Qiu, Jian Ma, R.F. Castañeda & X.G. Zhang, **sp. nov.** Fig. 2

MB 836852

Differs from *Heteroconium ponapense* and *H. indicum* by its shorter or smaller, 0-1-septate, dimorphic conidia that are obviously constricted at the septum.

Type: China, Jiangxi Province: Jinggangshan Mountain, Shuikou scenic spot, 26°33′03″N 114°07′55″E, on dead branches of an unidentified broadleaf tree, 6 Nov. 2014, J. Ma (**holotype**, HJAUP M0218).

Etymology: Latin, *simile*, referring to its similarity to *Heteroconium ponapense* and *H. indicum*.

Colonies on the natural substratum effuse, dark brown. Mycelium partly superficial, partly immersed, composed of branched, septate, pale brown, smooth hyphae. Conidiophores macronematous, mononematous, simple or branched, erect, straight or flexuous, cylindrical, smooth, brown to dark brown at the base, and paler towards the apex, 2–7-septate, 28–72 × 3–5 µm. Conidiogenous cells monoblastic, integrated, terminal, cylindrical, brown, smooth, 10–14 × 3.5–4.5 µm. Conidial secession schizolytic. Conidia acrogenous, blastocatenate, 11–15 × 4–5 µm, smooth, 0-1-septate, dimorphic, i) unicellular, ellipsoidal, fusiform, or ovoid, truncate at both ends or truncate at the base and rounded at the apex, concolorous, pale brown to dark brown,

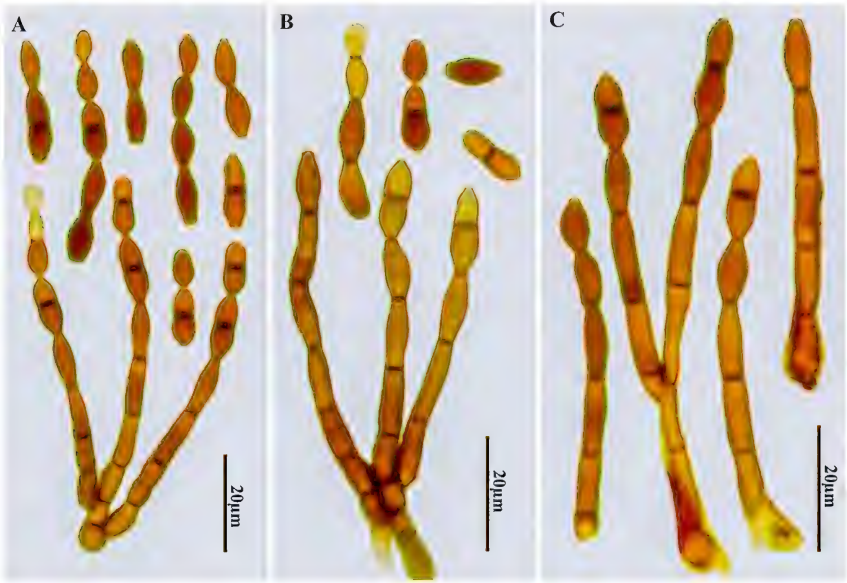


Fig. 2. *Heteroconium simile* (holotype, HJAUP M0218).
A–C. Conidiophores, conidiogenous cells, and conidia.

ii) bicellular, constricted at the septum, obcalceiform, ellipsoidal to obclavate, mostly bicolorous, basal cell brown or dark brown basal, apical cell pale brown or brown.

Comments –*Heteroconium* was erected by Petrak (1949) with *H. citharexyli* Petr. as type species. It is characterized by macronematous, mononematous, usually unbranched conidiophores, sometimes with a secondary branch originating after conidial secession or near percurrent regeneration. The conidiogenous cells are monoblastic, integrated, terminal, determinate or sometimes percurrent extended and produce acrogenous, blastocatenate, euseptate conidia that secede schizolytically (Petrak 1949; Ellis 1971; Castañeda & al. 1999, 2008; Taylor & al. 2001; Ma & al. 2012b).

Twenty-five *Heteroconium* epithets were listed in Index Fungorum (2020). However, *H. tetracoilum* (Corda) M.B. Ellis was transferred to *Lylea* Morgan-Jones due to its distoseptate conidia (Ellis 1976, Holubová-Jechová 1978). *Heteroconium solaninum* (Sacc. & P. Syd.) M.B. Ellis is not congeneric with *H. citharexyli;* it has an obligate association with asterinaceous fungi and

different conidial septation sequence and was designated as the type species of *Pirozynskiella* S. Hughes (Ellis 1976, Hughes 2007). *Heteroconium chaetospira* (Grove) M.B. Ellis, *H. eucalypti* Crous & M.J. Wingf., and *H. kleinzeense* Crous & Z.A. Pretorius were transferred to other genera (*Cladophialophora* Borelli; *Thyrinula* Petr. & Syd.; *Alysidiella* Crous) based on molecular phylogenetic analyses (Ellis 1976; Crous & al. 2006, 2007a,b, 2019; Cheewangkoon & al. 2012). Although *H. queenslandicum* Matsush. is a currently accepted name, this species exhibits a similar conidial ontogeny with *Parapleurotheciopsis* P.M. Kirk in its holoblastic conidia with an acropetal branched chain through an apical ramoconidium that secedes schizolytically (Matsushima 1989, MycoBank 2020); these characters were remarked by Castañeda-Ruíz & al. (2008). Thus, *Heteroconium* currently contains 19 species, among which 11 species have been recorded from China: *H. annesleae* S.C. Ren & X.G. Zhang, *H. arundicum* Chowdhry, *H. bannaense* J.W. Xia & X.G. Zhang, *H. decorosum* R.F. Castañeda & al., *H. fici* L.G. Ma & X.G. Zhang, *H. indicum*, *H. neolitseae* S.C. Ren & X.G. Zhang, *H. phellodendri* Jian Ma & X.G. Zhang, *H. ponapense*, *H. schimae* Y.D. Zhang & X.G. Zhang, *H. tsoongiodendronis* L.G. Ma & X.G. Zhang (Zhang & al. 2010; Ma & al. 2012a,b; Ren & al. 2012; Xia & al. 2012; Zhang 2018).

Among the 19 *Heteroconium* species, only *H. ponapense* Matsush. and *H. indicum* Varghese & V.G. Rao are resemble *H. simile* in having predominately 1-septate conidia. However, *H. ponapense* differs by its obclavate to ellipsoid, brown, longer (12–24 µm) conidia (Matsushima 1983), while *H. indicum* differs by its larger (23–42.5 × 8.5–10.5 µm), fusiform to cylindrical, pale brown to reddish brown conidia (Varghese & Rao 1980).

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# *Neosporidesmium himachalense* sp. nov. from India and *Neosporidesmina* gen. nov. to accommodate *N. micheliae*

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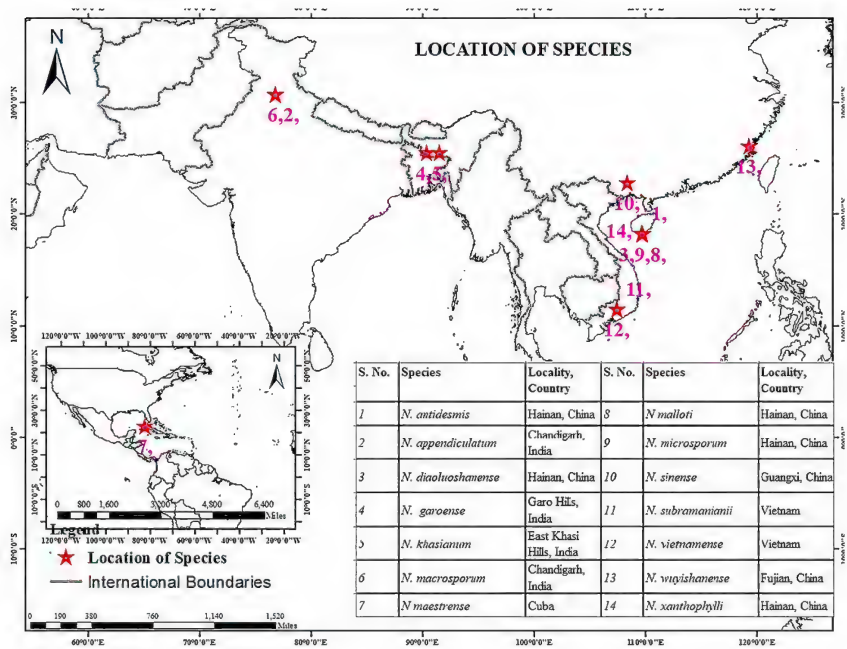
Abstract—A new species, *Neosporidesmium himachalense,* collected from a decaying twig of an unidentified angiosperm in Mandi Himachal Pradesh, India, is described and illustrated. It is characterized by macronematous, laterally and terminally diverging conidiophores, terminal, doliiform, monoblastic conidiogenous cells, and obclavate 7–18-distoseptate, pale brown conidia. A new genus *Neosporidesmina* is proposed for *Neosporidesmium micheliae*, which has euseptate conidia.

Key words—anamorphic fungi, hyphomycetes, taxonomy

## Introduction

Recent works on fungal biodiversity in India have prompted an increase in the mycobiota of the country with several new species and

new records (Adamčík & al. 2015; Buyck & al. 2017; Gautam &. al. 2020; Sushma & al. 2020; Verma & al. 2019, 2020). During continuing surveys of microfungi associated with plant materials in Himachal Pradesh, India, a synnematous fungus was collected on an unidentified angiosperm decaying twig. Its conidiogenesis and conidial morphology placed this fungus in *Neosporidesmium* Mercado & J. Mena. Fifteen *Neosporidesmium* species have already been reported, from four countries: China (8 spp.), Cuba (1 sp.), India (4 spp.), and Vietnam (2 spp.). Distribution is depicted in the map (Fig. 1) constructed using the geographical coordinates mentioned in the protologues of these taxa. The original *Neosporidesmium* generic concept was expanded by Zhang & al. (2011), after the description of *N. micheliae*, which is characterized by having euseptate conidia. This species is clearly separated from the distoseptate *Neosporidesmium* generic concept, and we propose a new genus, *Neosporidesmina*.




| S. No. | Species | Locality, Country | S. No. | Species | Locality, Country |
|---|---|---|---|---|---|
| 1 | *N. antidesmis* | Hainan, China | 8 | *N malloti* | Hainan, China |
| 2 | *N. appendiculatum* | Chandigarh, India | 9 | *N. microsporum* | Hainan, China |
| 3 | *N. diaoluoshanense* | Hainan, China | 10 | *N. sinense* | Guangxi, China |
| 4 | *N. garoense* | Garo Hils, India | 11 | *N. subramanianii* | Vietnam |
| 5 | *N. khasianum* | East Khasi Hills, India | 12 | *N. vietnamense* | Vietnam |
| 6 | *N. macrosporum* | Chandigarh, India | 13 | *N. wuyishanense* | Fujian, China |
| 7 | *N maestrense* | Cuba | 14 | *N. xanthophylli* | Hainan, China |

Fig. 1. Map showing the world distribution of *Neosporidesmium* spp.

## Materials & methods

Decaying culms, leaves, twigs, bark, and dead wood were collected in separate zip lock plastic bags and brought to the laboratory. The specimens were mounted on glass slides in either 4% KOH, 0.01% cotton blue or lactophenol (Kirk & al. 2008), The specimens were studied microscopically under a matrix stereo trinocular microscope (VL–Z60) and a compound microscope (VRS–2f) for macroscopic and microscopic characters. All the measurements were taken assisted by Pro MED software and represented as the extreme values without statistical treatment. The specimens were deposited in herbarium of Panjab University, Chandigarh, India (PAN).

Repeated attempts to culture the fungus failed on various media (i.e., 2% agar broth, cornmeal agar (CMA), potato dextrose agar (PDA), oatmeal agar, malt extract agar (MEA), and solidified glucose peptone medium comprising: glucose 10 g/l, peptone 2 g/l, $KH_2PO_4$ 1 g/l, $MgSO_4.7H_2O$ 0.5 g/l, agar 20 g/l in 1000 ml $H_2O$ with chips of host) with the addition of 50 µg/mL of two antibiotics (penicillin and streptomycin)—according to Choi & al. (1999) and Kirk & al. (2008). The distribution map was constructed by using ArcGIS software (Beyer 2004).

## Taxonomy

***Neosporidesmium himachalense*** Rajn.K. Verma, Prasher, Sushma, A.K. Gautam, Rajeshk. & R.F. Castañeda, **sp. nov.** Figs 2, 3

IF557994

Differs from *Neosporidesmium macrosporum* by its smaller conidia with fewer distosepta.

Type: India. Himachal Pradesh: Bilaspur (Jolplakhin), on dead and decaying twigs of angiosperm plant, 23 Nov 2015, R. K. Verma (**Holotype**, PAN 32815).

Etymology: Latin, *himachalense*, refers to the Himachal, where the type was collected.

Colonies on the natural substrate effuse, hairy, dark brown. Mycelium superficial, partly immersed in the substratum. Conidiomata synnematal, erect, cylindrical, scattered, dark brown to black, tapering toward the apex, up to 2400 µm high and 90–150 µm wide at the base. Conidiophores macronematous, unbranched, septate, smooth, brown to dark brown, diverging laterally and terminally. Conidiogenous cells monoblastic, doliiform, integrated, terminal, smooth, brown to dark brown, having 2–4 enteroblastic extensions (11.5–21 × 6.5–8.7 µm). Conidial secession schizolytic. Conidia 72.5–151.5 × 10.5–15.7 µm, solitary, acrogenous, obclavate, 7–18-distoseptate, smooth, pale brown and paler at the apex.

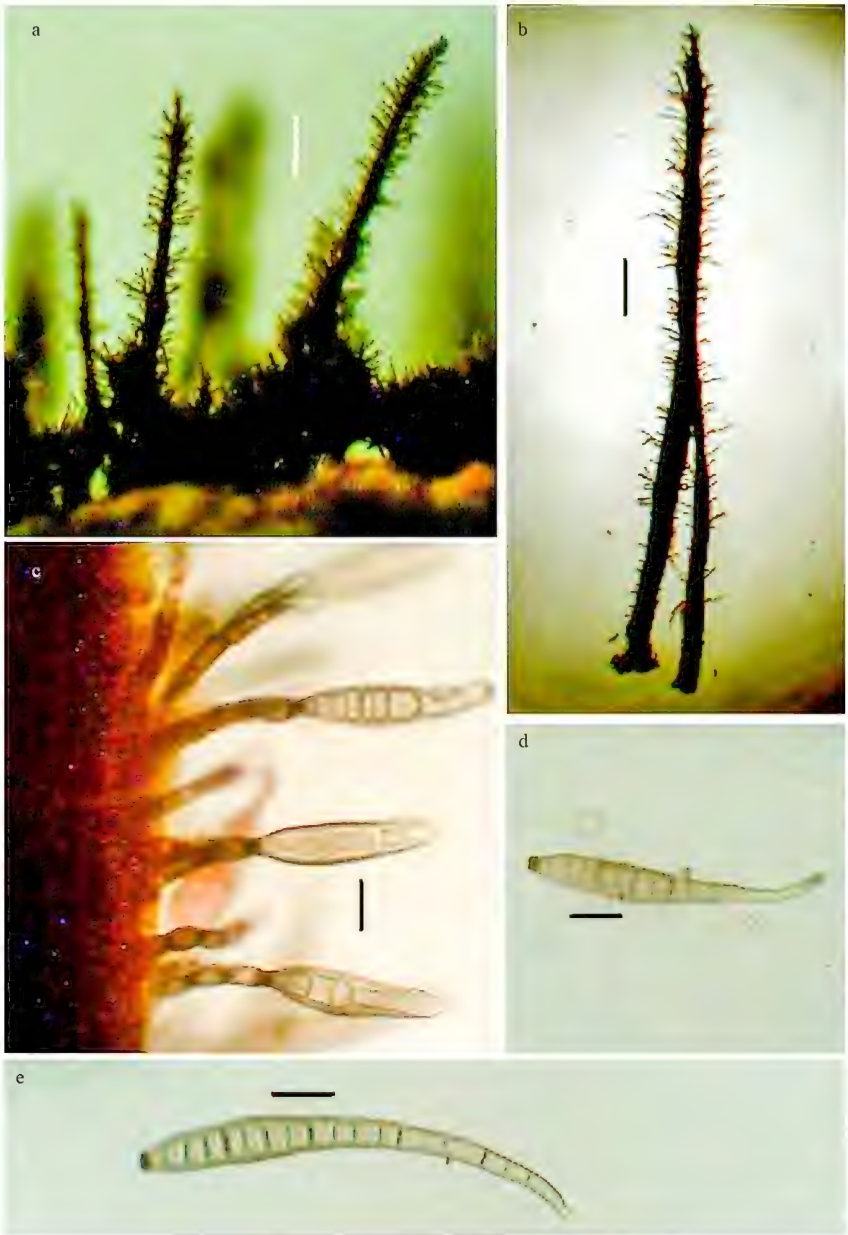



Fig. 2. *Neosporidesmium himachalense* (holotype, PAN 32815): A. Synnemata attached to natural substratum; B. Synnemata; C. Conidia attached to conidiogenous cells; D, E. Conidia. Scale bars: A = 100 mm; B = 200 µm; C–E = 20 µm.

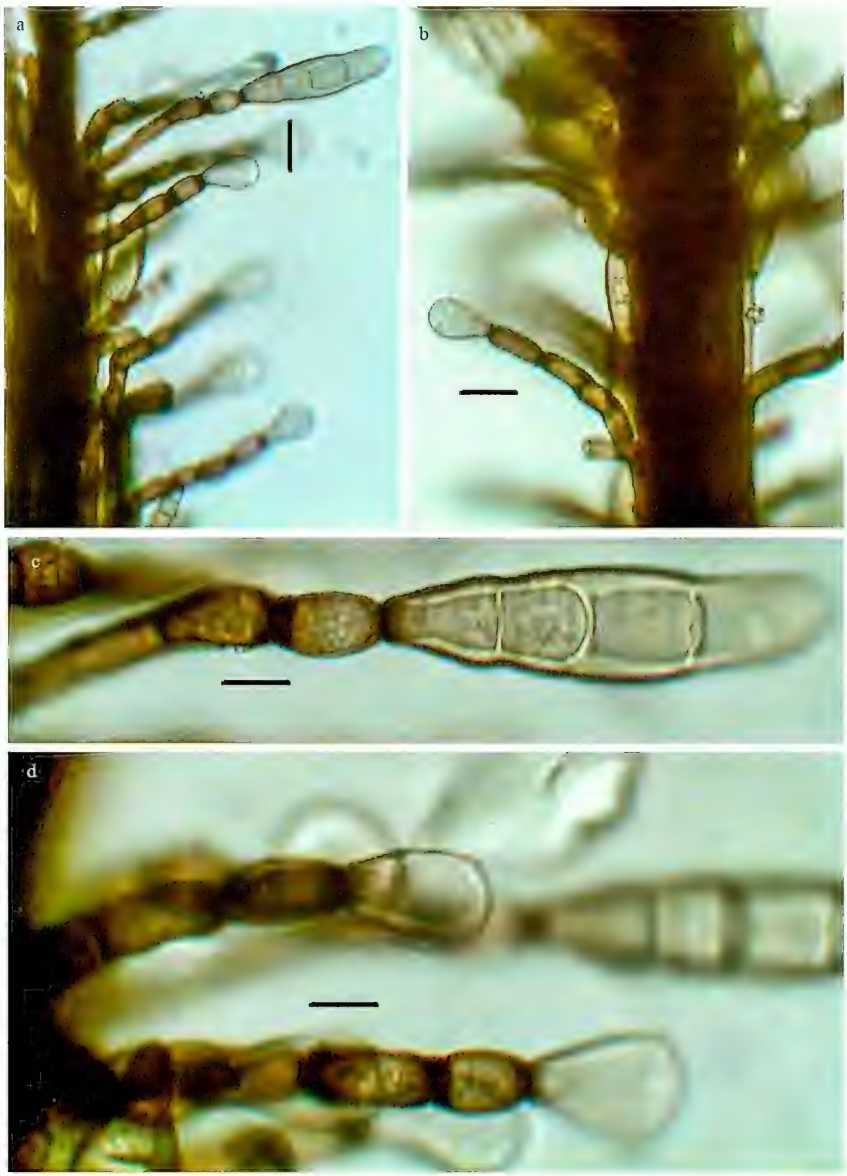



FIG. 3. *Neosporidesmium himachalense* (holotype, PAN 32815): Developing conidia and conidiogenous cells. Scale bars: A, B = 20 μm; C, D = 10 μm.

***Neosporidesmina*** R.F. Castañeda, Rajn.K. Verma, Prasher, Sushma, A.K. Gautam & Rajeshk., **gen. nov.**

IF 557995

Differs from *Sporidesmina* by its invariably euseptate conidia.

Type species: *Neosporidesmium micheliae* Y.D. Zhang & X.G. Zhang [≡ *Neosporidesmina micheliae* (Y.D. Zhang & X.G. Zhang) R.F. Castañeda & al.]

Etymology: Latin, *Neo-*, meaning, new + Latin, *sporidesmina*, referring to the genus *Sporidesmina*.

Colonies on the natural substrate effuse, hairy, dark brown. Mycelium superficial, partly immersed in the substratum. Conidiomata synnematal, scattered, erect, cylindrical, dark brown to black. Mycelium superficial and immersed. Conidiophores macronematous, unbranched, diverging laterally and terminally, septate, smooth, brown to dark brown. Conidiogenous cells monoblastic, cuneiform, cylindrical or subulate, integrated, determinate, terminal, smooth, brown to dark brown. Conidial secession schizolytic. Conidia solitary, acrogenous, obclavate or cylindrical, euseptate, smooth, brown.

***Neosporidesmina micheliae*** (Y.D. Zhang & X.G. Zhang) R.F. Castañeda, Rajn.K. Verma, Prasher, Sushma, A.K. Gautam & Rajeshk., **comb. nov.**

IF 557996

≡ *Neosporidesmium micheliae* Y.D. Zhang & X.G. Zhang, Sydowia 63(1): 128 (2011).

## Discussion

*Neosporidesmium* was erected by Mercado Sierra & Mena Portales (1988) for a single species, *N. maestrense*. The genus is characterized by effuse, dark brown to black, hairy colonies, unbranched conidiophores aggregating into erect, cylindrical, dark brown to black synnemata and integrated, terminal, monoblastic, lageniform or doliiform, determinate or percurrent extending conidiogenous cells. The conidia are solitary, obclavate or cylindrical, distoseptate, pale brown to brown, smooth. Subsequently, 14 other species were described: *N. antidesmatis, N. appendiculatum, N. diaoluoshanense, N. garoense, N. khasianum, N. macrosporum, N. malloti, N. micheliae, N. microsporum, N. sinense, N. subramanianii, N. vietnamense, N. wuyishanense,* and *N. xanthophylli* (Wu & Zhuang 2005; Ma & al. 2011; Zhang & al. 2011; Mel'nik & Braun 2013; Prasher & Verma 2015a,b; Li & al. 2015, 2017; Mel'nik & al. 2016, Pratibha & al. 2018; Index Fungorum 2020).

The criteria used for species delimitation are based primarily on conidiomata size, the enteroblastic extensions of conidiogenous cells, and

conidial morphology (Mercado Sierra & Mena Portales 1988, Wu & Zhuang 2005). Additionally, Prasher & Verma (2015b) described *N. appendiculatum* Prasher & Rajn.K. Verma as having conidial appendages. *Neosporidesmium macrosporum* Prasher & Rajn.K. Verma is comparable with *N. himachalense* but differs by its ≤1100 µm tall conidiomata and larger (108–250 × 12–16.5 µm) 9–20-distoseptate conidia. *Neosporidesmium himachalense* also differs from other species in size of synnemata and number of conidial distosepta and sizes (Table 1).

*Neosporidesmina* is close to *Sporidesmina* Subram. & Bhat, typified by *S. malabarica*, which is characterized by erect, carbonaceous, compact, unbranched or mostly branched, divergent, synnematal conidiomata formed by indistinguishable, unbranched, tiny packed, septate, pale to dark

Table 1. Morphological comparison of accepted *Neosporidesmium* species.

| Species | Synnemata [µm] | Conidial septa | Conidial size [µm] | Reference |
|---|---|---|---|---|
| *N. antidesmatis* | 950 × 30-50 | 11–15 | 48–75 × 7.5–9 | Ma & al. 2011 |
| *N. appendiculatum* | ≤4700 long | 9–15 | 82–148 × 17–19 | Prasher & Verma 2015b |
| *N. diaoluoshanense* | 605 × 40–60 | 8–11 | 105–135 × 10.5–13.5 | Li & al. 2015 |
| *N. garoense* | 700–1620 × 50–65 | 11–13 | 75–100 × 8–11 | Pratibha & al. 2018 |
| ***N. himachalense*** | 2400 × 90–150 | 7-18 | 72–151 × 10.5–15.7 | **This paper** |
| *N. khasianum* | 855–2750 × 50–60 | 10–12 | 103–135 × 10–18 | Pratibha & al. 2018 |
| *N. macrosporum* | 1100 × 80-140 | 9–20 | 108–250 × 12–16.5 | Prasher & Verma 2015a |
| *N. maestrense* | 2500 × 60-250 | 8–12 | 55–100 × 15–19 | Mercado Sierra & Mena Portales 1988 |
| *N. malloti* | 380 × 20-30 | 6–7 | 22.5–33.5 × 7–9 | Ma & al. 2011 |
| *N. microsporum* | 1200 × 20-40 | 6–7 | 45–67 × 6–7 | Wu & Zhuang 2005 |
| *N. sinense* | 1000 × 80-110 | 10-11 | 120–150 × 12–15 | Wu & Zhuang 2005 |
| *N. subramanianii* | 600–1200 × 45–80 | 8–15 | 80–120 × 10–14 | Mel'nik & al. 2016 |
| *N. vietnamense* | 500–1430 long | 9–12 | 75–110 × 11–13.5 | Mel'nik & Braun 2013 |
| *N. wuyishanense* | ≤1290 × 120 | 3–6 | 30–60 × 5–9 | Li & al. 2017 |
| *N. xanthophylli* | 1000 × 40-55 | 6–10 | 33.5–51.5 × 12–14 | Ma & al. 2011 |

brown conidiophores. The conidiogenous cells are monoblastic, cylindrical integrated, terminal, and producing solitary acrogenous elongated-fusiform brown conidia, that are 8–12-euseptate from the base toward near the end, but abruptly attenuate, sub-rostrate or subulate, hyaline, 1–2-distoseptate just below the apex (Subramanian & Bhat 1989). The two types of septa separate *Sporidesmina* from *Neosporidesmina*, which has only euseptate conidia.

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We are indebted to Dr. De-Wei Li (The Connecticut Agricultural Experiment Station, USA) and Dr. Rosa María Arias Mota (Instituto Tecnológico Superior de Xalapa, Mexico) for serving as pre-submission reviewers and to Dr. Lorelei L. Norvell for final editorial review and Dr. Shaun Pennycook for nomenclatural review. The IBP is thankful to the Ministry of Environment and Forests, Government of India, for financial assistance (vide letter 14/26/2008–ERS/RE dt. 06.07.2010), and to UGC (SAP, DRSIII). The authors (RKV, IBP and Sushma) are thankful to Chairperson Department of Botany Panjab University for providing infrastructural and laboratory facilities. RFCR is grateful to Cuban Ministry of Agriculture for facilities.

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# *Cladorrhinum yunnanense* sp. nov. from China

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Abstract—A new asexual fungus, *Cladorrhinum yunnanense*, isolated from unidentified fallen leaves of a broadleaf tree in Yunnan, China, is described by morphological characters and phylogenetic analyses of DNA sequence data from three loci (ITS, LSU and TUB).

Key words—*Lasiosphaeriaceae*, mitosporic fungi, *Sordariales*, taxonomy

## Introduction

*Cladorrhinum* Sacc. & Marchal was established in 1885 with *C. foecundissimum* Sacc. & Marchal as the type species (Marchal 1885). The genus is characterized by fast-growing colonies with pale to dark greenish, greyish, or brownish pigmentation, and conidiophores typically comprising intercalary phialides with lateral openings (pleurophialides), usually with a flaring collarette. The conidia are one-celled, hyaline or subhyaline, smooth-walled, commonly globose to dacryoid, and arranged in slimy masses. *Bahupaathra* Subram. & Lodha is synonymised with the earlier described *Cladorrhinum* (Mouchacca & Gams 1993).

[#] These authors contributed equally to this work.

Mouchacca & Gams (1993), who revised *Cladorrhinum*, accepted five species: *C. foecundissimum*, *C. brunnescens* W. Gams, *C. bulbillosum* W. Gams & Mouch., *C. phialophoroides* Mouch. & W. Gams, and *C. samala* (Subram. & Lodha) W. Gams & Mouch. Subsequently added to the genus were *C. flexuosum* Madrid & al., *C. microsclerotigenum* Madrid & al., *C. australe* Gasoni, *C. globisporum* Z.F. Zhang & L. Cai, *C. hyalocarpum* (Arx) X. Wei Wang & Houbraken, and *C. intermedium* (Stchigel & Guarro) X. Wei Wang & Houbraken (Madrid & al. 2011, Carmaran & al. 2015, Zhang & al. 2017, Wang & al. 2019). Wang & al. (2019) recently transferred *C. bulbillosum* to *Podospora* Ces. and *C. phialophoroides* to *Triangularia* Boedijn.

Fungal diversity in China is high but still mostly unexplored. Several new species from Yunnan Province have been previously described (Xia & al. 2012, 2014, 2016; Ma & al. 2014 a,b,c; Zhang & al. 2016; Guo & al. 2019; Zheng & al. 2019, 2020). During our ongoing survey of anamorphic fungi associated with plant leaves in tropical and subtropical forests of southern China, we collected a new species representing *Cladorrhinum.*

## Materials & methods

### Isolates and Morphological analysis

Diseased leaves were collected from an unidentified plant in Xishuangbanna, Yunnan Province, China. Tissue pieces (5 × 5 mm) were taken from the margin of leaf lesions and surface-sterilized by immersing in 75% ethanol solution for 1 min, 5% sodium hypochlorite solution for 30 s, and then rinsing three times in sterile distilled water for 1 min. The pieces were dried with sterilized paper towels and then placed on potato dextrose agar (PDA) (Cai & al. 2009). All the PDA plates were incubated at room temperature 25 °C for 2–4 d, and from which the peripheries of the colonies were picked out and inoculated onto new PDA plates. Colonies were photographed after 7 d and 15 d with a Powershot G7X mark II digital camera. Micromorphological characters were observed using an Olympus SZX10 stereomicroscope and Olympus BX53 microscope, both fitted with Olympus DP80 high definition colour digital cameras. All fungal strains were stored in 10% sterilized glycerin at 4 °C for further studies. The specimens are deposited in the Herbarium of Plant Pathology, Shandong Agricultural University, Taian, China (HSAUP). Ex-type cultures are deposited in the Shandong Agricultural University Culture Collection, Taian, China (SAUCC).

### DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from colonies grown on PDA, using the CTAB method (Doyle & Doyle 1990). The primer pair ITS1/ITS4 (White & al. 1990) was

Table 1. *Cladorrhinum* and related strains included in the phylogenetic analyses.

| Species name | Culture acc. no. | GenBank accession no. | | |
|---|---|---|---|---|
| | | ITS | LSU | TUB |
| *Cercophora ambigua* | CBS 215.60 | AY999137 | AY999114 | AY999147 |
| *Cercophora appalachianensis* | HKUCC3711 | AF177155 | — | — |
| *Cercophora areolata* | UAMH7495 | AY587911 | AY587936 | AY600252 |
| *Cercophora caudata* | CBS 606.72 | AY999135 | MH872289 | AY999151 |
| *Cercophora grandiuscula* | CBS 120013$^{T}$ | GQ922544 | KF557693 | — |
| *Cercophora samala* | CBS 109.93$^{T}$ | AY999134 | AY999111 | AY999140 |
| *Cercophora sulphurella* | SMH2531 | AY587913 | AY587938 | AY600254 |
| *Cladorrhinum australe* | INTA-AR 108 | KT321073 | KT312986 | KT291711 |
| | INTA-AR 110 | KT321074 | KT312987 | KT291712 |
| *Cladorrhinum brunnescens* | CBS 643.75$^{T}$ | NR137152 | FR692346 | — |
| *Cladorrhinum bulbillosum* | INTA-AR 102 | KT321068 | KT312982 | KT291706 |
| | CBS 304.90$^{T}$ | NR077199 | FR692339 | KT291720 |
| *Cladorrhinum flexuosum* | FMR 10415 | NR154757 | NG058773 | — |
| *Cladorrhinum foecundissimum* | BCCM 6980 | KT321080 | KT312993 | KT291721 |
| | CBS 180.66$^{T}$ | MH858767 | FR692343 | KT291717 |
| *Cladorrhinum globisporum* | CGMCC3.17921$^{T}$ | KU746680 | KU746726 | KU746771 |
| | CGMCC3.17922 | KU746679 | KU746725 | KU746772 |
| *Cladorrhinum hyalocarpum* | CBS 322.70$^{T}$ | MK926857 | MK926857 | MK926957 |
| | CBS 102198 | MK926858 | MK926858 | MK926958 |
| *Cladorrhinum intermedium* | CBS 433.96$^{T}$ | MK926859 | MK926859 | MK926959 |
| | CBS 100257 | MK926860 | MK926860 | MK926960 |
| *Cladorrhinum microsclerotigenum* | CBS 290.75$^{T}$ | NR154758 | FR692342 | — |
| *Cladorrhinum phialophoroides* | CBS 301.90$^{T}$ | NR077198 | FR692344 | KT291718 |
| *Cladorrhinum samala* | CBS 302.90 | KT321079 | KT312992 | KT291719 |
| | INTA-AR 168 | KT321078 | KT312991 | KT291716 |
| ***Cladorrhinum yunnanense*** | **SAUCC 0525** [HT] | **MN956794** | **MN956785** | **MT376683** |
| | **SAUCC 0791** | **MN956795** | **MN956786** | — |
| *Phialophora brevicollaris* | CBS 126.74$^{T}$ | NR121471 | — | GU727570 |
| *Podospora cochleariformis* | CBS 249.71 | MH860099 | AY999098 | AY999145 |
| *Podospora cupiformis* | CBS 246.71$^{T}$ | MH860098 | AY999102 | AY999149 |
| *Podospora curvicolla* | IFO 8548 | AY999122 | AY999099 | AY999148 |
| *Podospora decipiens* | CBS 258.69 | KX171946 | AY780073 | AY780130 |
| *Podospora didyma* | CBS 232.78 | AY999127 | AY999100 | AY999142 |
| *Podospora fibrinocaudata* | CBS 315.91$^{T}$ | MH862255 | MK926844 | MK926944 |
| *Podospora fimiseda* | CBS 990.96 | KX348039 | AY346296 | AY780133 |
| *Podospora intestinacea* | CBS 113106 | AY999121 | AY999104 | AY999152 |

used to amplify the rDNA internal transcribed spacer (ITS) region following White & al. (1990). The primer pair LR0R/LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994) was used to amplify a partial fragment of the large subunit (LSU) gene. The primer pair Bt2a/Bt2b (Glass & Donaldson 1995) was used to amplify the beta-tubulin (TUB) gene.

The DNA was amplified using an Eppendorf Master Thermocycler in 25 µL reaction volumes containing 12.5 µL Vazyme Green Taq Mix, 1 µL of each Biosune forward and reverse primer (10 µM), and 1 µL template genomic DNA adjusted with distilled deionized water to a total 25 µL volume. PCR parameters were 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C (ITS, LSU) or 60 °C (TUB) for 30 s, extension at 72 °C for 1 min, and a final elongation step at 72 °C for 10 min. PCR products were estimated visually by staining with GelRed after 1% agarose gel electrophoresis. Sequencing was done bi-directionally, conducted by the Biosune Company Limited. Consensus sequences were obtained using MEGA v. 7 (Kumar & al. 2016). All sequences generated in this study were deposited in GenBank (Table 1).

#### Sequence alignment and phylogenetic analysis

The quality of our amplified nucleotide sequences was checked and combined by MEGA v. 7 (Kumar & al. 2016), and reference sequences were retrieved from the National Center for Biotechnology Information (NCBI), based on recent publications on *Cladorrhinum* (Madrid & al. 2011, Carmarán & al. 2015, Zhang & al. 2017). Sequences were aligned using MAFFT v. 7.310 (Katoh & al. 2019; http://mafft.cbrc.jp/alignment/server/index.html) and manually corrected using MEGA v. 7.

The combined gene regions were phylogenetically analysed using Maximum-Likelihood (ML) and Bayesian Inference (BI) methods. RaxML (v. 8.2.9) and Bayesian analyses (MrBayes v. 3.2.6) were run on the CIPRES Science Gateway portal (Miller & al. 2012). Evolutionary models were calculated using MrModelTest v. 2.3 (Nylander 2004) selecting the best-fit model for each data partition according to the Akaike criterion. For ML analyses default parameters were used and bootstrap support (BS) used the rapid bootstrapping algorithm with the automatic halt option. Bayesian analyses included two parallel runs of 5,000,000 generations, with the stop rule option and a sampling frequency set to each 1000 generations. The 50% majority rule consensus trees and posterior probability (PP) values were calculated after discarding the first 25% of the samples as burn-in. The resulting trees were plotted using FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree) and edited with Adobe Illustrator CS v. 5. The individual gene datasets were assessed for incongruence before being concatenated by checking their individual phylogenies for conflicts between clades with significant ML and BI support (Mason-Gamer & Kellogg 1996, Wiens 1998).

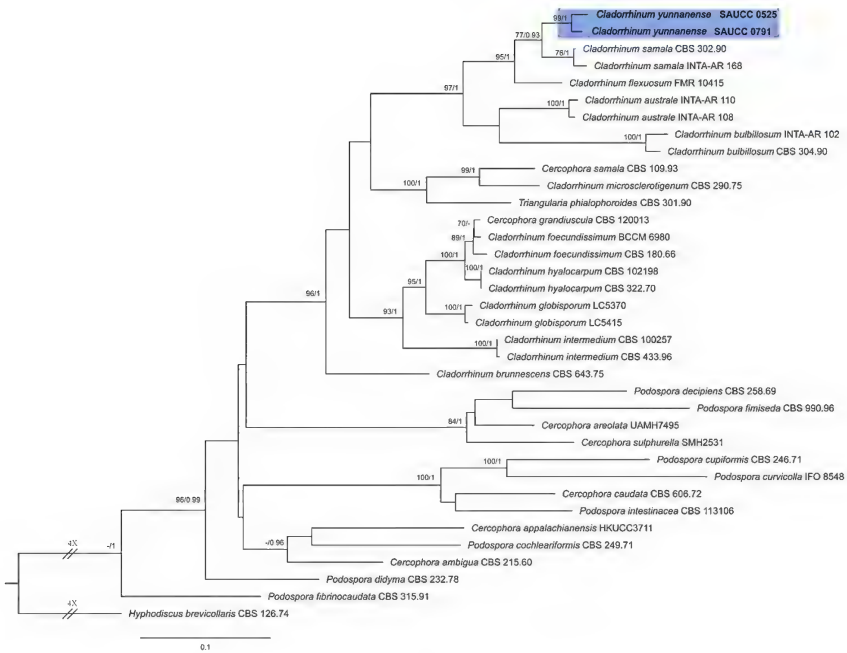


Fig. 1. The ML consensus tree inferred from the combined ITS, LSU and TUB sequence alignment. Branches are labeled with Maximum-Likelihood bootstrap support ≥75% and Bayesian posterior probabilities ≥0.95. The tree is rooted to *Hyphodiscus brevicollaris* (CBS 126.74). The scale bar indicates 0.1 expected changes per site.

## Phylogenetic results

The dataset comprised 36 taxa representing 28 species including *Hyphodiscus brevicollaris* (CBS 126.74) as the outgroup. The final alignment comprised a total of 2101 characters of the combined ITS, LSU, and TUB including gaps: ITS 1–545; LSU, 546–1357; and TUB, 1358–2101. Of these characters, 1315 were constant, 221 parsimony-uninformative, and 565 parsimony-informative. For ML and BI analyses, GTR+I+G for ITS and LSU, and GTR+G for TUB were selected and incorporated into the analyses. As the topology of ML tree confirmed the tree topologies obtained from the BI analyses, only the ML tree is presented (Fig. 1). In this tree, our strains formed a distinct clade and grouped together with *Cladorrhinum samala* and *C. flexuosum* with good support (BS = 95%, PP = 1). Therefore, we determined that our strains belonged to a novel species of *Cladorrhinum*.

## Taxonomy

***Cladorrhinum yunnanense*** Y. Pan, J.W. Xia, X.G. Zhang & Z. Li, **sp. nov.** Fig. 2

MB 836106

Differs from all other *Cladorrhinum* spp. by its blastic conidiogenous cell.

Type: China, Yunnan Province, Xishuangbanna, on unidentified fallen leaf of a broadleaf tree, 16 Apr. 2019, Y. Pan (**Holotype**, HSAUP 0525; ex-type living culture SAUCC 0525).

Etymology: in reference to the province containing the type locality.

Colonies on PDA pale grey to dark grey, cottony to fluffy, flat to slightly plicate, margin entire. Reverse pale brown to brown. Culture on OA white to grayish, margin entire, aerial mycelia sparse. Reverse white. Culture on MEA white to yellowish-brown, cottony to fluffy, margin filiform. Reverse white to yellowish. Vegetative hyphae hyaline, branched, septate, thin-walled, slightly rough, 1.5–4 µm diam. Microsclerotia not observed. Sporodochium forming on PDA within 30 d or longer, margin round, black, scattered over entire colony. Conidiophores single, unbranched, erect, straight or slightly flexuous, cylindrical, smooth, thick-walled, hyaline at the base, pale brown to brown in the upper part, 2–4-septate, 40–60 × 2–4 µm. Conidiogenous cell polyblastic, integrated, terminal, cylindrical, pale brown to brown, swollen at the apex, 15–25 × 2–4 µm. Conidia pale brown, smooth, thick-walled, oval to ellipsoid, aseptate, 6–10 × 2–4 µm, guttulate, aggregated in slimy head.

Additional specimen examined: **China, Yunnan Province, Xishuangbanna,** on unidentified fallen leaf of a broadleaf tree, 16 Apr. 2019, Y. Pan (HSAUP 0791; living culture SAUCC 0791).

Comments—According to Madrid & al. (2011) regarding convergent morphology in *Cladorrhinum*, characterization of any potentially new species within this genus should be included in the phylogenetic analyses. Based on a megablast search of GenBank nucleotide sequence database, the closest hit using the ITS sequence was *Cladorrhinum* sp. (strain RS_94, GenBank MK379585.1; Identities = 481/489 (98%), 3 gaps (0%)); the closest hit using the LSU sequence was *Cercophora costaricensis* (strain SMH4021, GenBank AY780059.1; Identities = 827/833 (99%), 1 gap (0%)), and the closest hit using the TUB sequence was *Cladorrhinum foecundissimum* (strain CBS 391.42, GenBank HQ877462.1; Identities = 282/300 (94%), 3 gaps (1%)).

*Cladorrhinum yunnanense* formed a well-supported sister clade (BS = 99%, PP = 1.0) to *C. flexuosum* and *C. samala*. Based on conidial

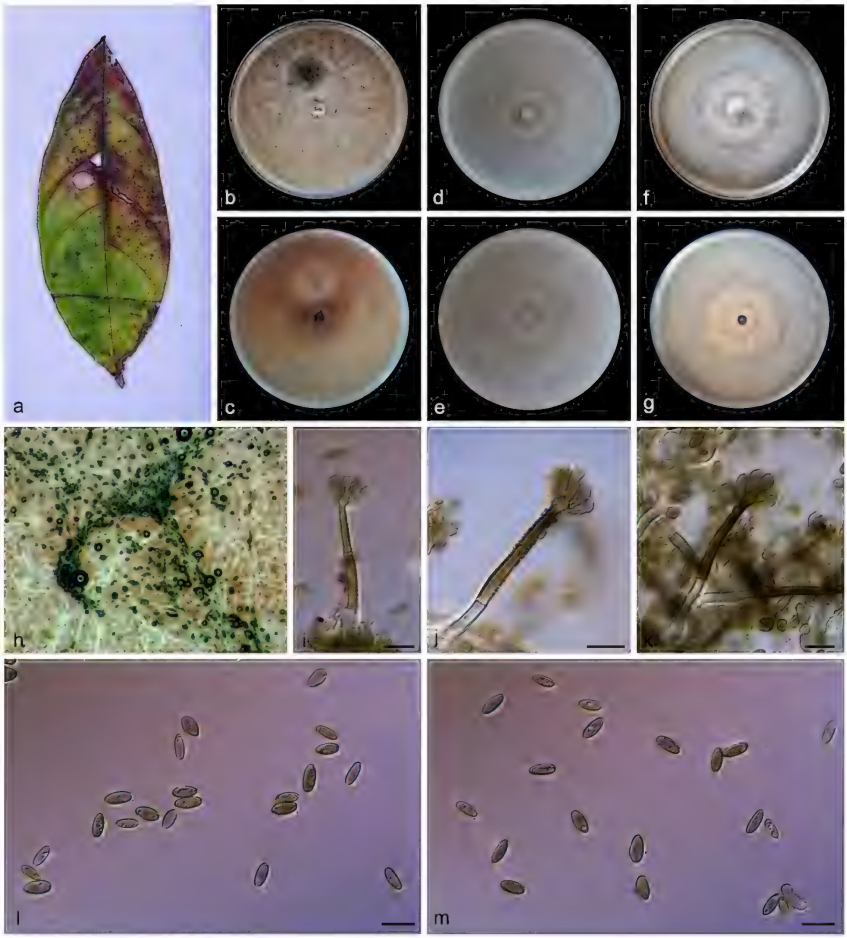


FIG. 2. *Cladorrhinum yunnanense* (holotype, HSAUP 0525). a. Diseased leaf; b,c. Colony on PDA; d,e. Colony on OA; f,g. Colony on MEA; h. Conidiomata; i-k. Conidiophores and conidia; l-m. Conidia. Scale bars: i-m = 10 μm

morphology, *C. yunnanense* (oval to ellipsoid, 6–10 × 2–4 μm) can be distinguished from *C. flexuosum* (mostly globose to dacryoid, 2–4 × 2–3 μm) and *C. samala* (dacryoid, 2–4 × 1.5–3 μm). *Cladorrhinum yunnanense* also differs from the previously described *Cladorrhinum* species by its blastic conidiogenous cell.

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# *Mazosia hainanensis* sp. nov. from tropical China

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Abstract—A new species, *Mazosia hainanensis*, is described from Hainan, China. It is most similar to *M. pseudobambusae* but differs by its yellowish green thallus and smaller ascospores (14–18.5 × 3–4 µm). An analysis of its relationships based on molecular phylogeny is given. A key to the *Mazosia* species recorded in China is also presented.

Key words—*Arthoniales,* lichenized fungi, *Roccellaceae,* taxonomy

## Introduction

*Mazosia* A. Massal. is a genus of lichenized fungi typified by *M. rotula* (Mont.) A. Massal. (Massalongo 1854), belonging to *Roccellaceae*, *Arthoniales*, *Arthoniomycetes* (Lücking 2008, Aptroot & al. 2014, Lücking & al. 2016). The status of this genus was in disarray until 1996, when Lücking & Matzer determined its position in *Roccellaceae* (Lücking & Matzer 1996). *Mazosia* is characterized by the rounded ascomata with three layers: excipulum layer with dark brown hyphae, a layer of crystals, and a thin thallus layer dominated by the photobiont. There are 8 ascospores per ascus that are hyaline, fusiform, and transversely septate. Conidia of two types are sometimes present (Santesson 1952, Lücking 2008, Lücking & al. 2016). According to Index Fungorum, *Mazosia* includes c. 34 species, most of which occur on leaves, except for four found on bark (Sparrius 2004, Aptroot & al. 2014).

Only six *Mazosia* species have been found in China, distributed in Guangdong, Hongkong, Sichuan, Taiwan, and Yunnan (Zahlbrückner 1930, Santesson 1952, Thrower 1988, Wei & Jiang 1991, Aptroot & Seaward 1999, Aptroot & Sipman 2001, Aptroot & al. 2003, Aptroot & Sparrius 2003).

Table 1. Specimens of *Mazosia* and related species and their LSU sequences used in the phylogenetic analysis.

| Species | Voucher specimen | GenBank No. |
|---|---|---|
| *Dendrographa alectoroides* | Ertz 12418 (BR) | HQ454527 |
| | Robertson 8970 (S-L63276) | EF081384 |
| *Dendrographa decolorans* | DUKE:0047570 | NG027622 |
| | UPS: Frisch 11/Se28 | KJ851054 |
| *Mazosia bambusae* | UPS: Joensson Guyana 3c | KJ851057 |
| *Mazosia carnea* | Ertz 15686 (BR) | KJ524309 |
| | Ertz 15684 (BR) | KJ524308 |
| *Mazosia melanophthalma* | UPS: Joensson Guyana 3b2 | KJ851063 |
| *Mazosia paupercula* | Ertz 9264 (BR) | KJ524310 |
| *Roccella fuciformis* | S-F206191 | KF036035 |
| | Tehler 8171 (L12540) | FJ638979 |
| *Tylophoron hibernicum* | Diederich 16335 | JF295084 |
| | Ertz 11546 (BR) | JF295083 |
| ***Mazosia hainanensis*** | HMAS-L 0145281 [T] | **MT683119** |
| | HMAS-L 0145282 | **MT683120** |

Note: Newly generated sequences are set in bold font.

## Materials & methods

Specimens. All materials were collected in Hainan Province, China, and are deposited in the Fungarium-Lichenes of the Institute of Microbiology, Chinese Academy of Sciences (HMAS–L). Morphological characters were examined and photographed under a LEICA M125 dissecting microscope. The anatomical characters were examined and photographed under Zeiss Axioscope2 compound microscope with a Zeiss Axio Imager A2 and an AxioCam MRc5 camera. Ascospores were tested using I (1% iodine solution). The lichen substances were detected using standardized thin layer chromatography (TLC) using solvent systems C and A (Orange & al. 2010).

DNA extraction, amplification, & sequencing. Genomic DNA was extracted from ascomata of the specimens followed a modified CTAB method (Rogers & Bendich 1988). The nuclear ribosomal RNA gene region, large subunit rDNA (LSU) was amplified using the LIC24R with LR7 primers (Frisch & al. 2014, Ertz & al. 2009). Reactions were carried out in 25 µL reaction system containing 1 µL each primer solution (10 µM), 2 µL genomic DNA, 8 µL $ddH_2O$, and 13 µL 2×Taq PCR

MasterMix®. Thermocycling conditions comprised: initial denaturation for 15 min at 95 °C; followed by 45 cycles of 45 s at 95 °C, 45 s at 53 °C, 1 min at 72 °C, and a final extension for 7 min at 72 °C. The target product of PCR was affirmed by electrophoresis on 1% agarose gels and sequenced by Majorbio (Shanghai).

Phylogenetic analysis. Due to lack of *Mazosia* ITS sequences, we chose LSU for sequence analysis. Our two newly generated LSU sequences were submitted to GenBank and aligned using MEGA (Kumar & al. 2016) with seventeen other sequences representing similar taxa in *Arthoniales* (Table 1), selected based on the results of LSU sequence Blast searches. *Tylophoron hibernicum* (D. Hawksw. & al.) Ertz & al., was chosen as outgroup (Urbanavichus & Urbanavichene 2017). The ML tree involving 1000 pseudoreplicates was generated by MEGA (Kumar & al. 2016), and the evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei 1993). The alignment was also subjected to a Neighbor-Joining analysis involving 1000 replicates with MEGA7 (Kumar & al. 2016), and the evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura & al. 2004).

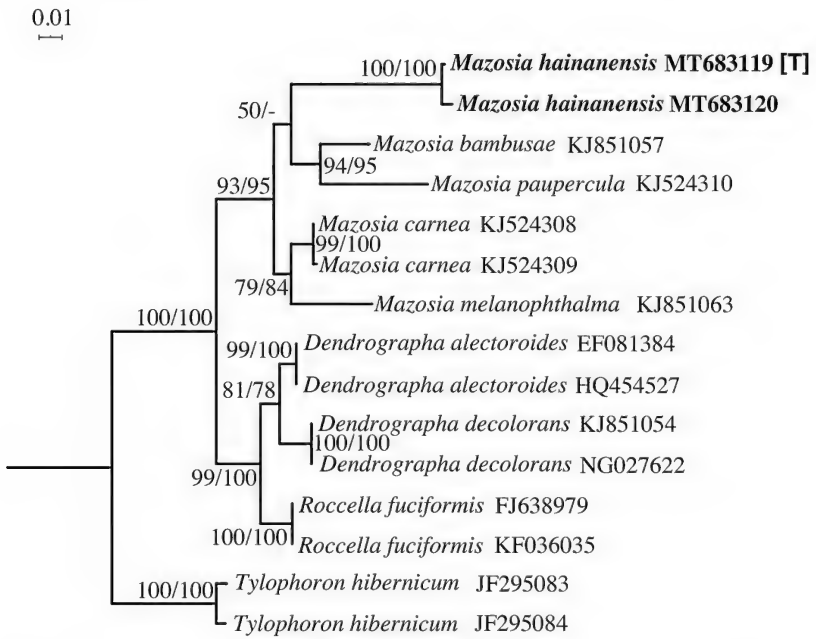


Fig. 1. Phylogenetic tree constructed from ML based on nrLSU sequences of *Mazosia* and related species. Maximum likelihood and Neighbor-Joining bootstrap support >50% are shown at nodes (ML/NJ). New sequences are set in bold font; other sequences were downloaded from GenBank. Genetic distance scale = 0.01 changes per site.

### Phylogeny

The phylogenetic tree constructed from ML based on the LSU sequences (1200 bp) is shown in Fig. 1. Other genera in *Roccellaceae—Dendrographa* Darb. and *Roccella* DC.—were included in the phylogenetic analysis. Within the well-supported (93/95) monophyletic five-species clade of *Mazosia*, the new species *M. hainanensis* formed an independent clade. *Mazosia bambusae* (Vain.) R. Sant. is placed as sister to *M. paupercula* (Müll. Arg.) R. Sant. with *M. carnea* (Eckfeldt) Aptroot & M. Cáceres shown as sister to *M. melanophthalma* (Müll. Arg.) R. Sant.

### Taxonomy

***Mazosia hainanensis*** Z.T. Yao, S.H. Jiang & Z.F. Jia, **sp. nov.** Fig. 2

MB 836195

Differs from *Mazosia pseudobambusae* by its yellowish green thallus and smaller ascospores.

Type: China, Hainan, Baoting County, Mount Qixian, on unidentified angiosperm leaf, 7 Sept. 2017, S.H. Jiang HN20171277 (**Holotype**, HMAS-L 0145281; GenBank MT683119).

Etymology: The epithet refers to the type locality province.

Thallus yellowish green, continuous, smooth, slightly shiny, often irregular in outline, finely verrucose, verrucae 20–30 µm tall. Photobiont cells rectangular, 6–15 × 2–4 µm, in radiate plates or slightly irregular. Ascomata rounded, 50–170 µm wide and 59–76 µm tall; disc black, translucent when moistened; margin slightly higher than disc and the disc is slightly lower than or as high as the thallus. Excipulum brown, 8–16 µm thick. Hypothecium 5–15 µm high, pale brown. Hymenium 42–50 µm high, colorless. Asci clavate, 34–45 × 10–12.5 µm. Ascospores fusiform, 3-septate, 14–18.5 × 3–4 µm, the second cell is slightly enlarged. Pycnidia low conical, 10–50 µm, Microconidia fusiform-ellipsoid, non-septate, 2–3.5 × 1–1.5 µm. Macroconidia not seen.

Chemistry: unidentified red fluorescent substance in the fourth and sixth zones in solvent system C and in the fourth and seventh zones in solvent system A.

Ecology & distribution: On unidentified angiosperm leaves in tropics; known only from the type locality, Hainan.

Additional specimen examined: **China**, **Hainan**, **Baoting County**, Mount Qixian, on unidentified angiosperm leaf, 7 Sept. 2017, S.H. Jiang HN20171184 (HMAS-L 0145282; GenBank MT683120).

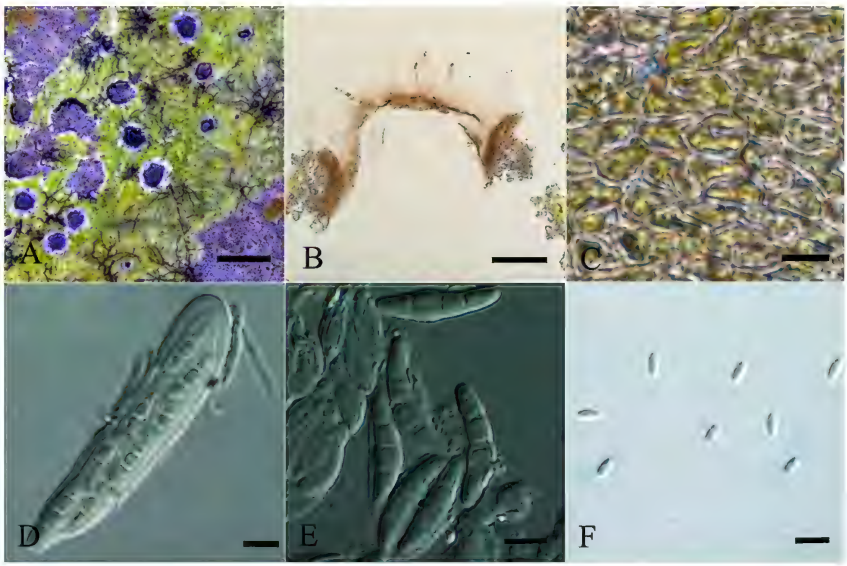


Fig. 2. *Mazosia hainanensis* (holotype, HMAS-L 0145281). A. Thallus with ascomata; B. Apothecium section; C. Photobiont; D. Ascus with ascospores; E. Ascospores; F. Microconidia. Scale bars: A = 0.2 mm; B = 50 μm; C–F = 5 μm.

Remarks: This species is characterized by its yellowish green, finely verrucose thallus, verrucae 20–30 μm, transversely septate ascospores 14–18.5 × 3–4 μm. *Mazosia pseudobambusae* Kalb & Vězda differs from the new species by its very pale brownish grey thallus, dark brown prothallus, and larger (20–28 × 4–5 μm) ascospores (Lücking 2008). The new species is also similar to *M. melanophthalma*, which differs by its larger (50–100 μm) verrucae, longer (15–22 μm) ascospores, and an ascomatal margin that always slopes outwards (Lücking 2008; Conran & Rogers 1983). Another related species is *M. dispersa* (J. Hedrick) R. Sant., distinguished by its less finely verrucose thallus and longer (26–39 μm) ascospores (Lücking 2008).

### Key to species of *Mazosia* known from China

1. Thallus glabrous ..... 2
1. Thallus with radiate ridges or finely to coarsely verrucose ..... 3
2. Ascospores 15–25 μm long ..... *M. phyllosema*
2. Ascospores 25–35 μm long, irregularly 3–7-septate, ..... *M. paupercula*

3. Thallus with radiate ridges, ascospores 3-septate, 14–22 × 4–6 µm ..... *M. rotula*
3. Thallus lacking radiate ridges, thallus verrucose ..... 4
4. Ascospores 5(–7)-septate, 26–39 µm long ..... *M. dispersa*
4. Ascospores 3-septate ..... 5
5. Verrucae 50–100 µm tall, ascospores 15–22 × 3–4 µm ..... *M. melanophthalma*
5. Verrucae 20–30 µm tall, ascospores 14–18.5 × 3–4 µm ..... *M. hainanensis*

## Conclusion

The morphological and molecular data demonstrate that the material from Hainan province represents a previously unknown foliicolous lichen that merits the status of a new species in *Mazosia*. The preliminary key is provided to the species of *Mazosia* currently known from China.

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# *Racocetra cromosomica* sp. nov. from Oaxaca, Mexico

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Abstract—Analysis of soil samples collected in the rhizosphere of *Agave karwinskii* in the Central Valleys of Oaxaca, Mexico, revealed an undescribed species of *Racocetra* that possesses ornamented spores. The ornamentation on the outer layer of the external spore wall consisted of rounded and elongated processes, which are organized in such a way that they look like chromosomes. Therefore, the new species was named *Racocetra cromosomica*. It is the first species of *Racocetraceae* (*Gigasporales*) described from Oaxaca, one of the Mexican states having the greatest floristic diversity.

Key words—arbuscular mycorrhizal fungi, *Glomeromycota*, mezcal, semiarid ecosystems, taxonomy

## Introduction

Mexico has a wide variety of ecosystems, and it is the center of origin, diversification and domestication of several plant species (Casas 2019). Oaxaca is one of the Mexican states characterized by the highest floristic diversity, comprising 8431 plant species (García-Mendoza & al. 2004). The diversity in Oaxaca arises from the convergence of five biogeographical provinces varying in climates, soils, and vegetation types (Morrone 2019), such as xerophilous scrublands and tropical dry forests, containing plants

of *Agavaceae*, a family endemic to America and one of ten monocot families with the highest species richness. In Oaxaca, the *Agave* genus is highly diverse, containing 32 of the 159 taxa distributed in Mexico (García-Mendoza & Galván 1995, García-Mendoza & al. 2004), all with great ecological, economic, and cultural importance for the inhabitants (Torres-García & al. 2019). Several *Agave* species are used to prepare mezcal, and people identify both agaves and mezcal as symbols of cultural identity. In preparing mezcal in the Central Valleys and the Sierra Sur of Oaxaca, people use *Agave vivipara* L. [= *A. angustifolia* Haw.], known in Mexico as agave espadín, and some wild species, as *Agave karwinskii* Zucc. (Ordoñez & Rodríguez 2008, García-Mendoza & al. 2004, 2017). Several *Agave* species are listed in the IUCN-Red List of Threatened species, including *A. karwinskii*, due to its limited distribution range (14,492 km$^2$) and the rapidly increasing demand for mature individuals for mezcal production (García-Mendoza & al. 2019).

Arbuscular mycorrhizal fungi (AMF) species (*Glomeromycota*) form mutualist symbiosis with almost 85% of land plant families, including *Agavaceae* (Carballar-Hernández & al. 2013, Goto & al. 2013; Chimal-Sánchez & al. 2018), and they promote the growth and nutrition for most plants (Smith & Read 2008), including *A. tequilana* F.A.C. Weber 'Azul' (Montoya-Martínez & al. 2019). Thus, in Mexico, AMF could be very important not only for conservation of floristic diversity but also for production of agave plants. In Mexico, currently 160 species of AMF are registered; however, the morphology of many AMF species found in the country suggests that they are undescribed (Varela & al. 2019, Polo-Marcial & al. 2021).

During a study on AMF associated with rhizosphere soil of mezcal agaves (*A. vivipara, A. karwinskii*) in the Central Valleys and Sierra Sur of Oaxaca, Mexico (the mezcal range), four new records and 48 morphospecies were registered (Chimal-Sánchez & al. 2018, Reyes-Jaramillo & al. 2019); however, ca. 30% of the AMF listed exhibit a morphology that does not fit those described species. Among them is a member of *Racocetraceae* (for this family see: Silva & al. 2012, Goto & al. 2012, Souza & al. 2018, Wijayawardene & al. 2020) that was registered in the rhizosphere of *A. karwinskii* and reported as *Racocetra* sp. 1 by Reyes-Jaramillo & al. (2019). Its subcellular spore structure, germination shield morphology, and histochemical properties indicates that it does belong in *Racocetra* (*Gigasporales*). Most importantly, spores of this fungus have a unique ornamentation consisting of chromosome-like outgrowths. Therefore, the fungus is here described as *Racocetra cromosomica*.

## Materials & methods

The Central Valleys (CV) and Sierra Sur of Oaxaca (SSO) are two of the eight economic regions of the State of Oaxaca. The CV and SSO belong to the "Sierra Madre del Sur" biogeographic province (Morrone 2019). The climate of the region is semi-dry, warm to semi-warm, with rains in summer. The soils according to World Reference Base for Soil Resources (WRB 2014) are Regosols and Leptosols, although Calcisols and Phaeozems are also present. The vegetation corresponds to tropical dry forests and xerophytic scrubland that have been strongly altered by agricultural activities, so that there are only fragments of this vegetation, with wild populations of *A. karwinskii* and cultivated fields with *A. vivipara* (INEGI 2010, Reyes-Jaramillo & al. 2019).

In the Central Valleys and Sierra Sur of Oaxaca, seven study sites (S) with wild populations of *A. karwinskii* were established in the municipalities of San Pedro Totolapan (S1), San Agustín Amatengo (S3, S4), Ejutla (S5), and San Luis Amatlán (S6, S9), while only two sites were established in fields cultivated with *Agave vivipara* in Ejutla (S2) and San Luis Amatlán (S7). The soils have a texture of Loamy sand (S1, S3–S9) or Sandy loam (S2), and pH ranging from neutral (S1, pH = 7.5; S2, pH = 7.19, and S3, pH = 7.28) to lightly alkaline (S4, pH = 7.86; S5, pH = 8.06; S6, pH = 8.02; S7, pH = 8.04; S8, pH = 7.96, and S9, pH = 8.0). On average, they are rich in organic matter (OM = 2.65% from S1–S4; OM = 4.9% from S5–S9), poor in available phosphorus ($PO_4$- Olsen = 1.6 mg $kg^{-1}$), and rich in extractable bases of calcium and magnesium, but low in potassium and sodium (Reyes-Jaramillo & al. 2019). However, our new *Racocetra* species was registered only in San Agustín Amatengo (S3) in soil with the highest percentage of sand (79%). Our new *Racocetra* coexisted with other AMF species, the most frequent of were *Acaulospora rehmii* Sieverd. & S. Toro, *A. scrobiculata* Trappe, *Cetraspora pellucida* (T.H. Nicolson & N.C. Schenck) Oehl & al., *Gigaspora gigantea* (T.H. Nicolson & Gerd.) Gerd. & Trappe, *Funneliformis halonatus* (S.L. Rose & Trappe) Oehl & al., *F. mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler, and *Racocetra fulgida* (Koske & C. Walker) Oehl & al. (Reyes-Jaramillo & al. 2019).

Pot trap cultures were established with native soil samples (600g per pot) and grown without fertilization in glasshouse conditions (Stutz & Morton 1996) using corn (*Zea mays* L.) and "guaje" (*Leucaena* sp., *Leguminosae*) as host plants. Host plants were grown for six months and watered every third day with distilled water, and then left to dry for two weeks to favor formation of AMF spores. Due to the small number of *Racocetra* spores obtained (1 spore per 300 g dry soil), only one single-species culture was set using two spores as inoculum and the same host plants. Unfortunately, this attempt failed, and no newly produced spores and mycorrhizal structures were found. Consequently, the new *Racocetra* species is here described based on field-collected spores and those extracted from trap cultures.

*Racocetra* spores were isolated by wet sieving and decanting (Gerdemann & Nicolson 1963), followed by centrifugation in 60% sucrose solution to eliminate organic matter and mineral remains. Several spores were then placed in Petri

dishes with distilled water, and their color was determined under a dissection microscope using the web color chart hosted by the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM 2018). Ten spores were mounted on microscopic slides in polyvinyl alcohol lacto-glycerol (PVLG) and in PVLG mixed with Melzer´s reagent 1:1 (v/v). Terminology for the species description, spore characteristics, and spore wall nomenclature are those adopted from Walker (1983) and Oehl & al. (2008). Likewise, we used an updated taxonomic key for *Racocetra* species proposed by Souza & al. (2018). Voucher specimens were deposited in the Herbarium of Instituto Politécnico Nacional, Mexico City, Mexico (ENCB), the Herbarium of Universidad Autónoma de Tlaxcala, Tlaxcala state, Mexico (TLXM), and the Laboratory of Legume Biosystematics, Department of Biology, Universidad Autónoma Metropolitana-Iztapalapa, Mexico City, Mexico (UAMI).

## Taxonomy

***Racocetra cromosomica*** Chim.-Sánch., Varela & Montaño, **sp. nov.** FIGS 1, 2

IF 557326

Differs from all other *Racocetra* and gigasporalean species by its unique ornamentation formed on the outer spore wall, consisting of rounded and elongated processes resembling condensed chromosomes.

TYPE—Mexico, Oaxaca, Central Valleys in San Agustín Amatengo, 16°31′06″N 96°47′08″W; isolated from the rhizospheric soil of *Agave karwinskii* growing within a xerophytic scrubland (SAASM) and coming from a propagation pot (SAASM-Prop) with *Zea mays* and *Leucaena* sp. (guaje) as trap plants; isolated June 2017, E. Chimal-Sánchez (**Holotype**, ENCB121802 [SAASM-Prop-slide-001]; **isotypes**, TLXM-5034 [SAASM-Prop-slide-002], UAMI-002 [SAASM-Prop-slide-003]).

ETYMOLOGY—referring to the distinctive ornamentation (rounded and elongated processes) on the outer spore wall that resembles chromosomes.

SPOROCARP formation is unknown. Spores are formed singly in the soil and terminally on a bulbous sporogenous cell. Spores are red-brown (20-80-60-0) to dark red-brown (60-80-100-0); globose, 368.6-445.3 µm.

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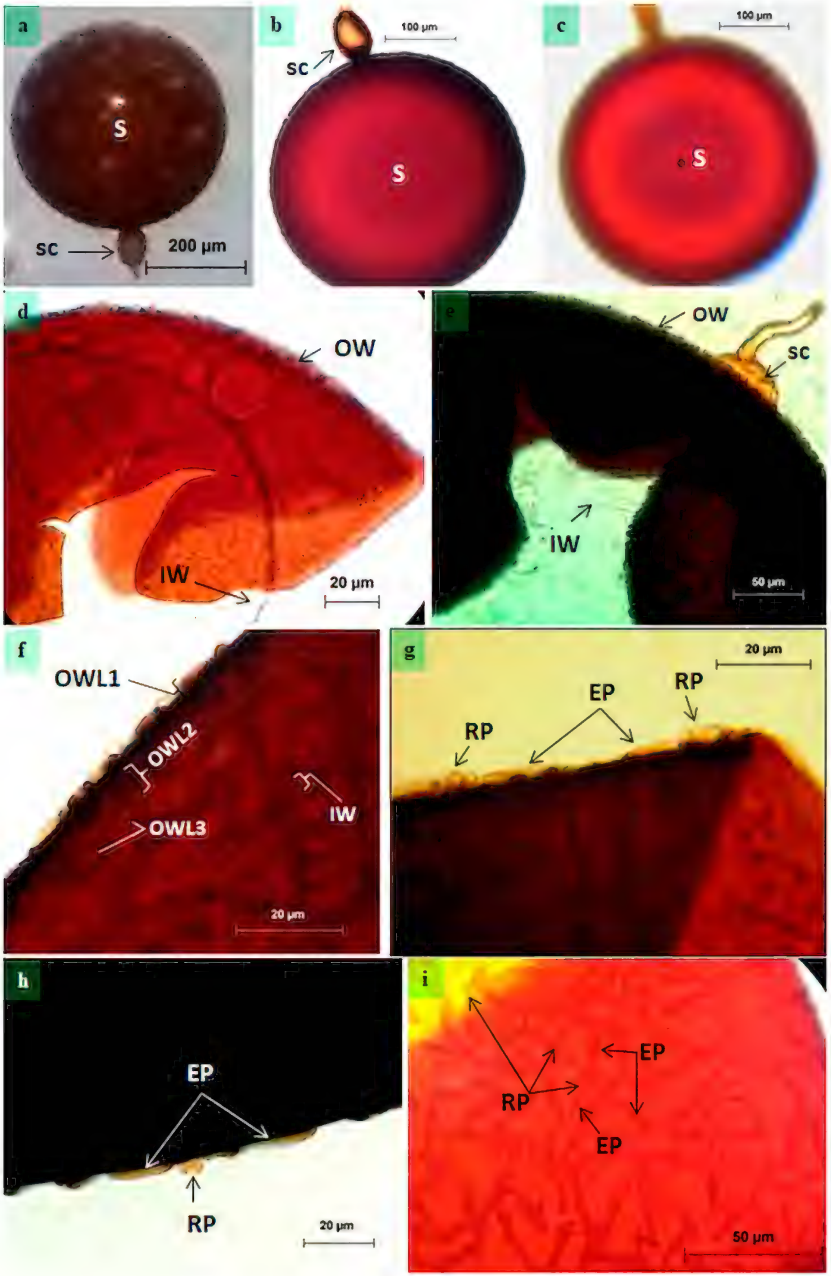
FIG. 1. *Racocetra cromosomica* (holotype, ENCB121802): a, b. Spore (S) in water (a) and PVLG (b) with bulbous sporogenous cell (sc) characteristic of the *Gigasporales*; c. The spore surface of an intact spore ornamented with elongated processes seen in plan view; d. Outer spore wall (OW) separated from inner spore wall (IW) of a crushed spore; the unique chromosome-like processes ornamenting the upper surface of the outer spore wall are visible; e. Outer (OW) and inner (IW) spore wall and bulbous sporogenous cell with no reaction in Melzer´s reagent; f. Outer spore wall with three permanent layers (OWL1-3), of which OWL1 is ornamented, and inner spore wall (IW); g, h. The upper surface of layer 1 of the outer spore wall ornamented with rounded (RP) and elongated (EP) processes; i. The distribution and arrangement of rounded (RP) and elongated (EP) processes (chromosomes-like) on the spore surface.


a
S
sc
200 µm
b
100 µm
sc
S
c
100 µm
S
d
OW
IW
20 µm
e
OW
sc
IW
50 µm
f
OWL1
OWL2
OWL3
IW
20 µm
g
20 µm
EP
RP
RP
h
EP
RP
20 µm
i
EP
RP
EP
50 µm

Spore wall comprising two walls: an outer wall (OW) and an inner wall (IW). OW has three closely adherent layers (OWL1, OWL2, and OWL3) of a total thickness of (15.4–)16.6(–17.6) µm. OWL1 is a permanent rigid layer, yellow-brown (0-10-40-0) to dark red-brown (60-80-100-0). The surface of this layer has double ornamentation resembling condensed chromosomes when observed in plan view. The first ornamentation type comprises rounded processes, (1.9–)3.7(–5.4) µm high and (3.8–)4.8(–5.7) µm wide at their base. Regularly, these processes are arranged in groups of 2, 4, 6, 8, or more, and when grouped they have a width of (11.1–)11.9(–12.7) µm and a height of (4.9–)5.1(–5.4) µm. The second type of ornamentation consists of elongated outgrowths, which are fused to the rounded ones and 1, 2, 3 or 4 elongated processes may be attached. In addition, the elongated processes may be connected to another group of rounded processes forming an irregular reticulum when the spores are observed in plan view. The elongated processes are claviform, (10.5–)16.4(–21.2) µm long, (4.4–)5.2(–6.5) µm wide, and (2.8–)3.4(–4.3) µm high. Both types of ornamentations are yellow-brown (0-10-40-0) to dark red-brown (60-80-100-0). The second layer of the spore wall (IWL2) is laminated, (9.1–)10.3(–12.4) µm thick, smooth, and hyaline (20-0-20-0). OWL3 is hyaline, often difficult to observe as it closely adheres to OWL2, <1 µm thick. None of the spore wall layers stained in Melzer´s reagent.

Inner Wall has two adherent layers (IWL1 and IWL2) that are semi-flexible, hyaline (30-0-20-0), jointly <5 µm thick, and do not stain in Melzer´s reagent.

Germination Shield formed on the outer surface of IW. It is hyaline (20-0-20-0), oval, (149.7–)165.5(–181.7) µm long × (102.4–)126.5(–140.9)

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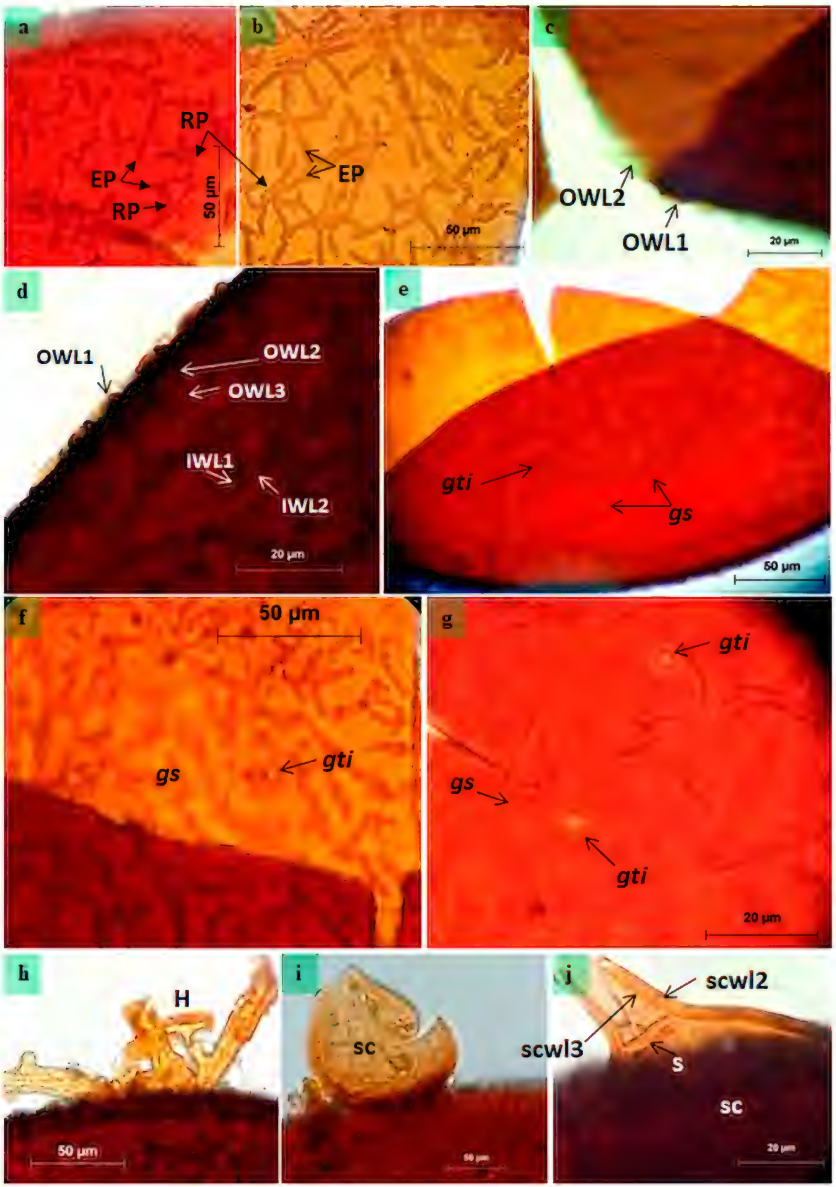
Fig. 2. *Racocetra cromosomica* (isotype, TLXM-5034): a, b. The arrangement and shapes of rounded (RP) and elongated (EP) processes (chromosomes-like) on layer 1 of the outer spore wall from spores crushed in Melzer´s reagent; c. Ornamented, dark-colored layer 1 (OWL1) and smooth, colorless layer 2 (OWL2) of the outer spore wall; d. Three-layered outer spore wall (OWL1-3) and two-layered inner spore wall (IWL1-2); e-g. Spores with germination shields (gs) composed by multiple small compartments (6–8), each with generally one germ tube initiations (gti); h. Germination from gti; note the thick and reddish brown color of the hyphae (H); i, j. Subglobose to ovoid sporogenous cell (sc) with a septum (S) at its base; note sc has no ornamentations and its wall consists of only two layers (scwl2 and scwl3) continuous with outer spore wall layers 2 and 3.


a
RP
EP
RP
50 µm
b
EP
50 µm
c
OWL2
OWL1
20 µm
d
OWL1
OWL2
OWL3
IWL1
IWL2
20 µm
e
gti
gs
50 µm
f
50 µm
gs
gti
g
gti
gs
gti
20 µm
h
H
50 µm
i
sc
j
scwl2
scwl3
S
sc
20 µm

µm wide, multilobed (6-8), each lobe generally with one germ tube initial (gti) from where germination tubes emerge.

Bulbous sporogenous cell formed terminally from an extraradical mycorrhizal hypha, yellow brown (20-40-100-0), sub-globose to ovoid, (56.1-)61.3(-63.9) µm wide and (68.9-)74.6(-77) µm long in the region from the spore base to the subtending hypha septum. Sporogenous cell wall smooth with two layers (scwl2, scwl3) continuous with OWL2 and OWL3. Scwl1 is absent since the bulb does not have the ornate layer of the spores (OWL1), scwl2 is yellow-brown (20-40-100-0), 2-3 µm thick, and scwl3 is yellow (0-30-80-0), 1.1-1.4 µm thick.

Auxiliary cells were not observed.

Distribution & ecology — *Racocetra cromosomica* is known only from Central Valleys, San Agustín Amatengo, Oaxaca, Mexico. The spores of *R. cromosomica* have been recorded only from soil associated with the rhizosphere of *A. karwinskii* growing in a xerophilous scrubland with remnants of other *A. karwinskii* wild populations. Soil characteristics are loamy sandy (79% sand), $pH_{H2O}$ = 7.2, soil organic matter = 3.2%, available phosphorus ($PO_4^-$-Olsen) = 0.7 mg $kg^{-1}$, Ca = 15 $cmoles_{(+)}$ $kg^{-1}$, Mg = 7.5 $cmoles_{(+)}$ $kg^{-1}$, K = 0.29 $cmoles_{(+)}$ $kg^{-1}$, and Na = 0.14 $cmoles_{(+)}$ $kg^{-1}$ (Reyes-Jaramillo & al. 2019). *Racocetra cromosomica* probably has preference for sandy soils like other *Gigasporales* (Lekberg & al. 2007; Vieira & al. 2020).

Mycorrhizal associations unknown. In the AMF multi-species propagation pot, *R. cromosomica* was propagated with maize (*Zea mays*) and "guaje" (*Leucaena* sp.) as host plants, but its sporulation was very low (one spore per 300 g dry soil). However, the presence of spores in these cultures suggested that *R. cromosomica* formed arbuscular mycorrhiza, as do other *Racocetra* species grown in single-species cultures.

## Discussion

The formation of spores from a bulbous sporogenous cell, the subcellular structure of spores containing two spore walls, the features of the spore germination shield, and the lack of staining reaction of the inner spore wall in Melzer's reagent unambiguously support the fungus collected by us in Mexico and here described as a new species of *Racocetra*.

The ornamentation of the upper surface of *Racocetra cromosomica* spores, consisting of rounded and elongated processes that are organized as if they were chromosomes, is a unique and very important diagnostic character that easily distinguishes this species from other *Racocetra* species and species

of other gigasporalean genera producing ornamented spores. Spores of *R. undulata* T.C. Lin & C.H. Yen are much smaller (195–225 µm vs. 368.6–445.3 µm) and are ornamented with pit undulations (Lin & Yen 2011), and the spore ornamentation in *R. persica* (Koske & C. Walker) Oehl & al., consists of fine spines (Oehl & al. 2008, Souza & al. 2018). *Racocetra minuta* (Ferrer & R.A. Herrera) Oehl & al., has a double ornamentation on the outer spore wall, but this ornamentation is composed of projections with central depressions (Oehl & al. 2008, Souza & al. 2018). *Intraornatospora intraornata* Goto & al., (≡ *R. intraornata* B.T. Goto & Oehl) produces ornamented gigasporoid spores, but the ornamentation consists of densely packed tubes that, importantly, occur on the lower surface of a laminate layer of the outer spore wall (Goto & al. 2009, 2012).

The only *Racocetra* species that have spores ornamented with processes (outgrowths resembling warts) are *R. beninensis* Oehl & al., *R. verrucosa* (Koske & C. Walker) Oehl & al., *R. coralloidea* (Trappe & al.) Oehl & al., *R. gregaria* (N.C. Schenck & T.H. Nicolson) Oehl & al., and *R. crispa* F.A. Souza & al. However, the spores of both *R. beninensis* and *R. verrucosa* are lighter in color and ornamented with processes that are smaller (0.9–2.1(–2.8) µm long, 0.9–3.8 µm wide in *R. beninensis*; 0.5–1.5 µm long, 0.5–1.5 µm wide in *R. verrucosa*) and simpler in construction (Koske & Walker 1985, Tchabi & al. 2009). *Racocetra coralloidea* spores are ornamented with flattened warts with angular margins (most 2–12 µm wide × 1–3 µm high) and those of *R. gregaria* with rounded warts (most 11–26.7 µm wide × 3–7 µm high) that differ in size and are clearly separated from each other, never occurring in groups as in *R. cromosomica* (Oehl & al. 2008, Souza & al. 2018). The ornamentation of *R. crispa* clearly differs from that of *R. cromosomica* by displaying multiple cloud-like projections with a basal diameter of (5–)15–41 µm and a height of (5.2–)9.5–18 µm (Souza & al. 2018).

Other species of the *Gigasporales* that can be morphologically confused with *R. cromosomica* due to the formation of similarly colored ornamented spores are *Scutellospora dipapillosa* (C. Walker & Koske) C. Walker & F.E. Sanders, *Dentiscutata colliculosa* B.T. Goto & Oehl, *D. nigerita* Khade, *D. nigra* (J.F. Redhead) Sieverd. & al., and *D. reticulata* (Koske & al.) Sieverd. & al. Apart from morphological differences in the spore ornamentation, these species also differ from *R. cromosomica* by possessing three spore walls instead of two (Koske & Walker 1985, Goto & al. 2010, Khade 2010, Oehl & al. 2008).

Although *R. cromosomica* sporulated in multi-species trap cultures, the low numbers of spores obtained did not allow us to conduct molecular and phylogenetic analyses to determine the position of this species among sequenced *Racocetra* spp. Nonetheless its spore morphology both clearly

supports its novelty and easily distinguishes *R. cromosomica* from other ornate species in the *Gigasporales*.

We are aware that *R. cromosomica* should be characterized molecularly in the future to track occurrence elsewhere based on environmental studies. Finally, the fact that *R. cromosomica* has so far been found only in the rhizosphere of *A. karwinskii*, a highly vulnerable species endemic to Mexico, suggests that *A. karwinskii* is critical for in situ conservation of this mycorrhizal fungus and that this new *Glomeromycota* species could be essential in the recovery of *A. karwinskii* populations within mezcal producing regions in Oaxaca, Mexico, particularly in the town of San Agustín Amatengo.

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# *Bactrospora cozumelensis* sp. nov. from Mexico

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Abstract—*Bactrospora cozumelensis* is described as a new species associated with mangrove forests in Cozumel Island, Mexico. The specimens were collected on *Rhizophora mangle* bark in Punta Sur Ecological Park, Cozumel Island Biosphere Reserve, Quintana Roo, Mexico. This species is characterized by an erumpent to sessile apothecium, laterally carbonized excipulum, and 3-septate ascospores that are slightly constricted in the middle.

Key words—*Arthoniales,* lichens, *Roccellaceae*, taxonomy

## Introduction

*Bactrospora* A. Massal. comprises 37 species of crustose lichenized fungi that grow mainly on the bark of trees (Wijayawardene & al. 2018, Van den Boom & al. 2018, Herrera-Campos & al. 2019). The genus is widely distributed in both tropical and temperate areas but often goes unnoticed (Sobreira & al. 2015). It is characterized by a thallus that is poorly developed, crustose, and associated with photobionts of *Trentepohliaceae*, bitunicate asci with an apical KI+ blue apparatus, and ascospores that are cylindrical to acicular and multiseptate (Egea & Torrente 1993). *Bactrospora* differs from similar genera such as *Lecanactis* or *Opegrapha* by its cylindrical to acicular and multiseptate ascospores, its apical ascal apparatus with a conspicuous KI+ blue ring, its pruinose thallus or disk, and its ascomatal shape (Egea

& Torrente 1993). *Bactrospora* displays four ascospore types (*dryina*-type, *patellarioides*-type, *homalotropa*-type, and *jenikii*-type) and its asexual form produces pycnidia that are immersed or subimmersed in the thallus or substratum and produce variably shaped conidia (Egea & Torrente 1993).

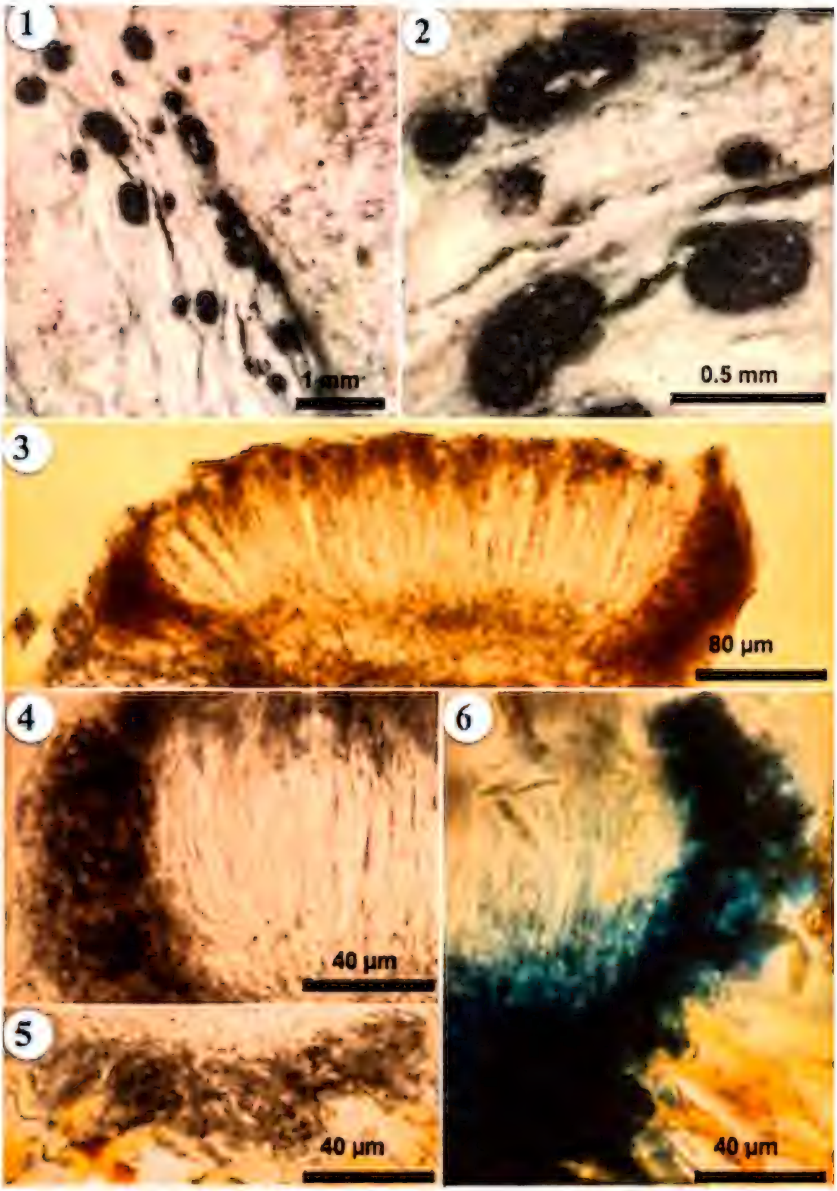
The genus was initially placed in the *Bactrosporaceae* by Rabenhorst (1869: 37, 60, as "*Bactrosporeae*") and this was accepted by Eriksson (1981). Subsequently, it was regarded as a synonym of the *Opegraphaceae* (Cannon & al. 1985, Eriksson & Hawksworth 1991), and later Hawksworth & al. (1995) and Grube (1998) included it in *Roccellaceae* (*Arthoniales*) based on macro- and micromorphological and chemical features. Recent phylogenetic studies treat *Bactrospora* as a genus incertae sedis within *Arthoniales* (Lücking & al. 2016, Wijayawardene & al. 2018).

In the Americas, 17 *Bactrospora* species have been reported, mostly from coastal ecosystems (Egea & Torrente 1993, Egea & al. 2004). Although Mexico is a megadiverse country, the lichen biota is insufficiently studied, and only two *Bactrospora* species have been reported: *B. jenikii* (Vězda) Egea & Torrente from Quintana Roo (Guzmán-Guillermo & al. 2019) and *B. lecanorina* Herrera-Camp. & al. from Jalisco (Herrera-Campos & al. 2019); both species were discovered in tropical forests.

During a mycological survey of mangrove on Cozumel Island, we collected specimens belonging to this genus that could not be assigned to any published species and so propose a new species here.

## Materials & methods

The mangrove forests lie within Cozumel Island Biosphere Reserve, in the Punta Sur Ecological Park, 20°16′41″–18′27″N 86°58′05″–87°00′41″W, covering 1114 ha at 0–7 m a.s.l. The climate is tropical with an average temperature of 25°C and a mean annual precipitation of 1570 mm (annual maximum of 250 mm occurring in October and minimum of 40 mm occurring in April; INEGI 2013). The mangrove forests include *Rhizophora mangle* L., *Laguncularia racemosa* (L.) C.F. Gaertn., *Conocarpus erectus* L., and *Avicennia germinans* (L.) L. (Téllez-Valdez & al. 1989). The specimens were collected in Punta Sur Ecological Park on 18 May 2019 on *R. mangle* bark and are deposited in fungal collection "Dr. Gastón Guzmán Huerta" at the Herbarium Escuela Nacional de Ciencias Biológicas of Instituto Politécnico Nacional, Mexico City, Mexico (ENCB) and the Herbarium in Universidad Veracruzana, Xalapa, Veracruz, Mexico (XALU). Latitude/longitude coordinates were obtained with GPS eTrex (Garmin). Morphological examinations were conducted as outlined by Brodo & al. (2001). The specimens were morphologically examined using a Carl Zeiss Primo Star microscope, and hand-cut sections were examined under a Carl Zeiss model Stemi Dv4 stereoscope. Ascospore measurements were made in water




Figs 1–6: *Bactrospora cozumelensis* (holotype, ENCB—García & Bravo 31a). 1. Gregarious young and mature apothecia on dead wood; 2. Apothecia mature, discoid; 3. Apothecium in transverse section; 4. Excipulum in lateral section; 5. Under side of subhymenium; 6. Excipulum after KI.

at 1000× magnification and only well-developed ascospores lying outside the asci were measured. Amyloidy was tested with Lugol´s solution before and after pretreatment with KOH 5%. Spot tests were made using standard methods (Brodo & al. 2001). A thin-layer chromatography (TLC) was performed with solvent C (Elix 2014). Measurements of micromorphological characters were taken from rehydrated tissues in water. Terminology is based on Egea & Torrente (1993).

## Taxonomy

***Bactrospora cozumelensis*** Y. García, Guzm.-Guill., R. Valenz. & Raymundo, **sp. nov.** FIGS 1–11

MB 836514

Differs from *Bactrospora incana* by its smaller ascospores, its epruinose apothecia, and its corticolous habit.

TYPE: México, Quintana Roo, municipality of Cozumel, Punta Sur Ecological Park, 20°18′00″N 87°00′36″W, 0 m a.s.l., mangrove forest in a coastal lagoon, on bark of *Rhizophora mangle* (*Rhizophoracaeae*), 18 May 2020, Y.A. García & M.A. Bravo 31a (**Holotype**, ENCB).

ETYMOLOGY: *cozumelensis* refers to the type locality.

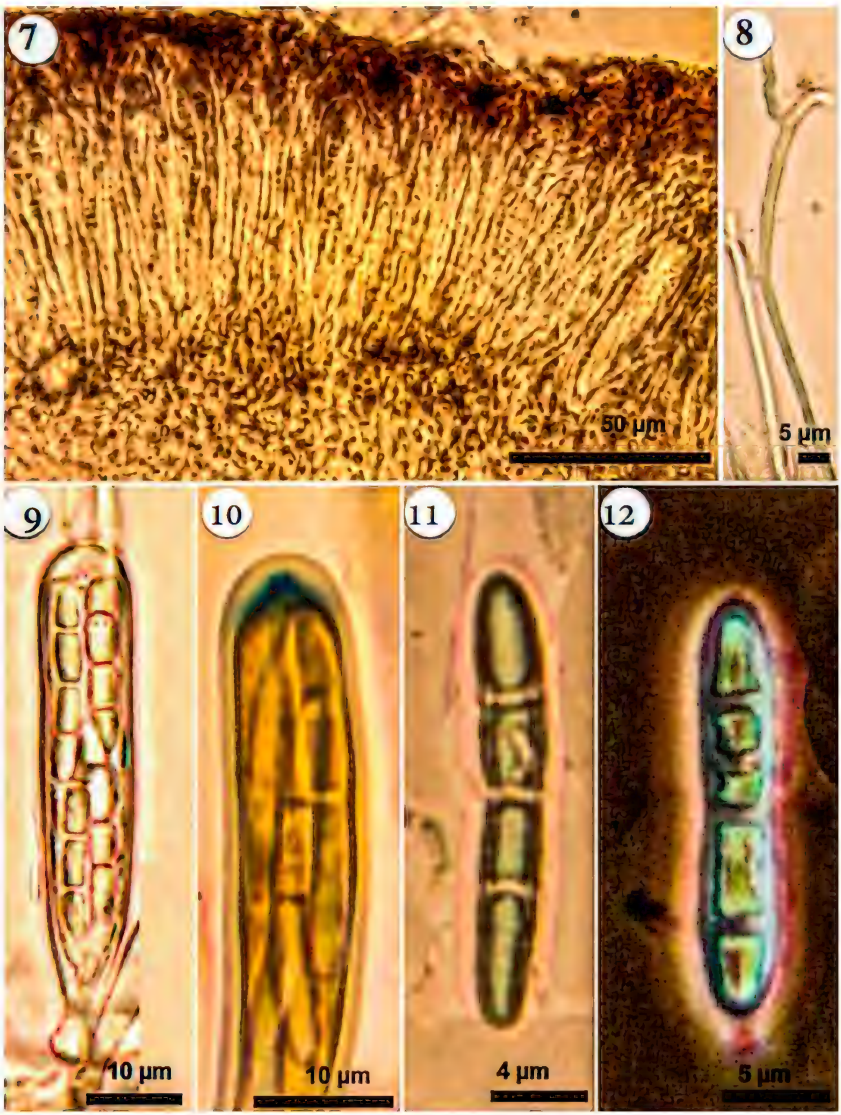
THALLUS crustose, continuous, effuse, thin, whitish, slightly shiny, surrounded by a black prothallus line; I–, K/I–, and Cl–; trentepohlioid ellipsoid cells inside of the thallus; APOTHECIA 0.1–0.3 mm diameter, roundish, dark, with thin margin to immarginate distinguishable for being darker, epruinose, initially erumpent on the bark, turning sessile at maturity, numerous, scattered in the thallus; EXCIPULUM 25–30 µm at the top and 40–60 µm at the base, laterally carbonized, open below the subhymenium, I–, K/I+ deep blue; HYMENIUM (85–)130–200(–230) µm, not inspersed, I+ reddish, K/I–; SUBHYMENIUM 100–130 um, hyaline I–, K/I+ blue. Paraphyses, long, branched, forming a reticulate, light brown pseudoepithecium without pruinose granules, K+ dark olive, I– and KI–; ASCI (40–)45–52(–55) × 8–10 µm (n = 20), cylindrical, hyaline, bitunicate, stipulated; ASCOSPORES eight per asci, (15–)17–20(–23) × (2–)3–4 µm (n = 35), of *jenikii*-type, hyaline, with 3(–4) transverse septa, slightly constricted in the middle.

PYCNIDIA not seen.

CHEMISTRY: K–, C–, UV–. TLC: no substances detected.

ADDITIONAL SPECIMEN EXAMINED: **MÉXICO, QUINTANA ROO, Municipality of Cozumel**, Punta Sur Ecological Park, 20°18′00″N 87°0′36″W, 0 m a.s.l., mangrove forest in a coastal lagoon, on bark of *Rhizophora mangle,* 18 May 2020, Y.A. García & M.A. Bravo 32 (XALU).

ECOLOGY & DISTRIBUTION: Growing on primary branch bark of *Rhizophora mangle* in a mangrove forest situated in a coastal lagoon; known only from




Figs 7–12: *Bactrospora cozumelensis* (holotype, ENCB—García & Bravo 31a). 7. Hymenium in water; 8. Paraphyses; 9. Asci in water; 10. Asci after KI; 11, 12. Ascospores in 5% KOH.

the type locality. *Bactrospora cozumelensis* was found growing together with other lichens including *Pyrenula cerina* Eschw., *Coniocarpon cinnabarinum* DC., and *Arthonia antillarum* (Fée) Nyl.

Comments—*Bactrospora cozumelensis* is diagnosed by apothecia that are subimmersed when immature and sessile when mature and its 3(–4)-septate ascospores that are slightly constricted in the middle. The similar species *Bactrospora brevispora* R.C. Harris is distinguished by its sessile apothecia, an excipulum closed below the subhymenium, and larger (20–32 × 3–3.5 µm) ascospores (Harris 1990; Egea & Torrente 1993). *Bactrospora incana* Egea & Torrente, which also resembles *B. cozumelensis,* differs by its larger (28–42 × 4.5–6.5) ascospores, densely hairy young ascomata, and epilithic habitus (Egea & Torrente 1993) (Table 1). Neither *B. brevispora* nor *B. incana* have been reported in mangrove forests.

Table 1. Comparisons between *Bactrospora cozumelensis* and related species.

| Character | *B. brevispora* | *B. cozumelensis* | *B. incana* |
|---|---|---|---|
| Unicellular hairs on thallus | Absent | Absent | Present |
| Ascomata size (mm) | 0.3–0.8 | 0.1–0.3 | 0.2–0.4 |
| Excipulum opening below subhymenium | Closed | Open | Open or reduced to a thin band |
| Ascus size (µm) | (45–)50–70 × 10–12 | (40–)45–52(–55) × 8–10 | 60–75 × 18–21 |
| Ascospore type | *jenikii*-type | *jenikii*-type | *jenikii*-type |
| Ascospore size (µm) | 20–32(–35) × 3–3.5(–4) | (15–)17–20(–23) × (2–)3–4 | 28–42 × 4.5–6.5 |
| Number of ascospore septa | 3–7 | 3–4 | 3–7 |
| Habitus | Epiphyte | Mangrove epiphyte | Epilithic |
| Distribution | USA, Jamaica (Egea & Torrente 1993) | Mexico (this publication) | Venezuela (Egea & Torrente 1993) |

In the Americas, only three other species of *Bactrospora* show the *jenikii*-type ascospores produced by *B. cozumelensis: B. brevispora, B. incana,* and *B. jenikii;* of these, *B. jenikii* with 8–13 septa is the most distinct (Sobreira & al. 2015) while *B. cozumelensis* has the smallest *jenikii*-type spores. Of the three *Bactrospora* species reported in Mexico, only *B. cozumelensis* is found in mangrove forest; *B. lecanorina,* found in a dry tropical forest in Jalisco, has *patellarioides*-type ascospores with more septa (Herrera-Campos & al 2019), and *B. jenikii,* found in sub-deciduous forest in Bacalar lagoon, has larger ascospores (Guzmán-Guillermo & al. 2019).

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# *Lembosia mimusopis sp. nov.* from Thailand

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Abstract—A novel species *Lembosia mimusopis* is introduced with evidence from morpho-molecular characterization. It was collected from the leaves of *Mimusops elengi* in Chiang Rai Province, Thailand. The new species is unique in having a mucilaginous sheath surrounding its immature ascospores. LSU sequence analyses phylogenetically support separation of this species from other *Lembosia* species.

Keywords—*Asterinaceae*, *Asterinales*, *Dothideomycetes*, *Lembosiaceae*, *Sapotaceae*

## Introduction

Léveillé (1845) established *Lembosia* with four species: *Lembosia tenella* Lév. (type species), *L. dendrochili* Lév., *L. drimydis* Lév., and *L. macula* Lév.

Hansford (1946) included *Lembosia* in his new family *Asterinaceae* Hansf. Based on morphology, Hosagoudar & al. (2001) and Hosagoudar (2012) excluded *Lembosia* from *Asterinaceae* and treated the genus as the type of *Lembosiaceae* Hosag However, Hongsanan & al. (2014) re-instated *Lembosia* within the *Asterinaceae* based on molecular data derived from *L. albersii* Henn. Dai & al. (2018) revealed that two species, *L. albersii* and *L. xyliae* Zeng & al., are phylogenetically separate from *Asterinaceae* but retained them in the same order, *Asterinales*. Three other species (*L. syzygii* Sivan. & R.G. Shivas, *L. abaxialis* Firmino & R.W. Barreto, *L. brigadeirensis* Firmino & al. clustered with *Asterina* species in a distinct clade and Dai & al. (2018) treated them in *Asterinales* sensu lato. There are about 200 *Lembosia* species epithets listed in Index Fungorum (2019). The type species *L. tenella* has not yet been sequenced, and only five *Lembosia* species have been sequenced.

*Lembosia* species are considered as host-specific on plants in tropical and sub-tropical regions and are usually identified based on association with their host family or genus (Müller & von Arx 1962, Sivanesan & Shivas 2002). For instance, Sivanesan & Shivas (2002) introduced *L. syzygii* from the leaves of *Syzygium suborbiculare* (Benth.) T.G. Hartley & L.M. Perry and *Araucaria heterophylla* (Salisb.) Franco. The inherent morphological characters of *Lembosia* are oval, elongated thyriothecia with an X- or Y-shaped or longitudinal dehiscence, lateral appressoria on the hyphae, and ovate to globose asci containing 4–8 brown uniseptate ascospores (Hosagoudar & Goos 1991, Hosagoudar 2012). The asexual morph of this genus is undetermined.

In this study, we introduce a novel species, *Lembosia mimusopis,* which was collected from the leaves of *Mimusops elengi* in Thailand. Morphological characters of the new taxon and its taxonomic placement supported by phylogenetic analysis are provided.

## Materials & methods

### Sample collection, morphological studies, specimen deposition

Fresh leaf specimens were collected from Mae Fah Luang Botanical Gardens, Chiang Rai, Thailand, in December 2018. Specimens were examined using a Motic SMZ 168 Series microscope. Hand sections of the fruiting structures were mounted in water and 5% KOH for microscopic studies and photomicrography. Blue ink was used to stain the mucilaginous sheath around the ascospores. The micro-morphology was examined using a Nikon ECLIPSE 80i compound microscope and photographed using a Canon 750D digital camera fitted to the microscope.

Measurements were made with the Tarosoft (R) Image Frame Work program and figures were processed with Adobe Photoshop CS3 Extended version 10.0 software. Specimens were deposited in the Mae Fah Luang University Herbarium, Chiang Rai, Thailand (MFLU). The new taxon was linked with Facesoffungi and Index Fungorum databases as explained in Jayasiri & al. (2015) and Index Fungorum (2019).

Table 1. Taxa used in the phylogenetic analyses and their GenBank accession numbers and voucher numbers

| Species | Voucher | GenBank (LSU) |
|---|---|---|
| *Asterina cestricola* | TH 591 | GU586215 |
| *Asterina chrysophylli* | VIC 42823 | KP143738 |
| *Asterina cynometrae* | MFLU 13-0373 [T] | NG057120 |
| *Asterina fuchsiae* | TH 590 | GU586216 |
| *Asterina magnoliae* | MFLU 16-0072 | MG844186 |
| *Asterina melastomatis* | VIC 42822 [T] | NG057055 |
| *Asterina phenacis* | TH 589 | GU586217 |
| *Asterina siphocampyli* | PPMP 1324 | HQ701140 |
| *Asterina weinmanniae* | TH 592 | GU586218 |
| *Asterina zanthoxyli* | TH 561 | GU586219 |
| *Asterotexis cucurbitacearum* | PMA M-0141224 [T] | HQ610510 |
| *Batistinula gallesiae* | VIC 42514 | KP143736 |
| *Buelliella physciicola* | Ertz 18113 | KP456147 |
| *Buelliella poetschii* | Ertz 18115 | KP456149 |
| *Hemigrapha atlantica* | Ertz 14014 | KP456151 |
| *Inocyclus angularis* | VIC 39748 | KP143732 |
| *Karschia cezannei* | Ertz 19186 [T] | NG060323 |
| *Karschia talcophila* | Ertz 16749 | KP456155 |
| *Labrocarpon canariense* | Ertz 16907 | KP456158 |
| *Lembosia abaxialis* | VIC 42825 [T] | NG060317 |
| *Lembosia albersii* | MFLU 13-0377 | KM386982 |
| *Lembosia brigadeirensis* | VIC 44208 | MF664531 |
| ***Lembosia mimusopis*** | **MFLU 19-1225 [T]** | **MN563123** |
| *Lembosia syzygii* | MFLU 13-0633 | MG844185 |
| *Lembosia xyliae* | MFLU 14-0004 [T] | NG059589 |
| *Melaspileella proximella* | G.M. 2015-04-29 | KY654747 |
| *Melaspileopsis diplasiospora* | Ertz 16624 | KP456165 |
| *Parmularia styracis* | VIC 42447 [T] | NG060137 |
| *Prillieuxina baccharidincola* | VIC 42817 | KP143735 |
| *Stictographa lentiginosa* | Ertz 17570 | KP456170 |

[T] = Ex-types and authentic strains. Newly generated sequences are indicated in **bold.**

### DNA extraction, PCR amplification, sequencing

Genomic DNA was extracted directly from fresh thyriothecia using a Forensic Genomic DNA Extraction Kit.

The total volume of PCR mixture (25 µL) contained double distilled water ($ddH_2O$) (11 µL), 2× QinKe PCR Master Mix (11 µL), DNA template (1 µL) and 10 µm of each primer (1 µL). The complete 28S large subunit rDNA (LSU) gene was amplified using LR0R/LR5 primers (Vilgalys & Hester 1990). PCR amplification conditions consisted of an initial denaturation step of 5 min at 94 °C and final extension step of 10 minutes at 72 °C. For the LSU amplification, the 37 cycles consisted of denaturation at 94 °C for 1 minute, annealing at 54 °C for 50 seconds and elongation at 72 °C for 1 minute. PCR products were viewed on 1% agarose electrophoresis gels, stained with ethidium bromide (Thambugala & al. 2015). The amplified PCR products were sent to Qinke, a commercial sequencing provider in Kunming, China.

### Sequence alignment & phylogenetic analyses

Newly generated sequences were assembled using Seqman and subjected to the standard BLAST search (https://blast.ncbi.nlm.nih.gov) to identify the closest matches in GenBank. The accession numbers of taxa used in our analyses are shown in Table 1. Sequences (LSU) were aligned using MAFFT v. 6.864b (Katoh & al. 2017) and manually adjusted in BioEdit v. 7.0 (Hall 2004).

Maximum likelihood analysis was performed using RAxML on XSEDE in CIPRES (Miller & al. 2010, Silvestro & Michalak 2012). The optimal ML tree was obtained with 1000 separate runs under the GTR+GAMMA substitution model resulted from model tests. Bayesian inference (Larget & Simon 1999) was performed using the MrBayes 3.2.2 on XSEDE tool in CIPRES (Ronquist & al. 2012). Posterior probabilities (PP) were obtained from Markov chain Monte Carlo sampling (MCMC) (Rannala & Yang 1996, Ronquist & al. 2012) when the average standard deviation of split frequencies fell below 0.01. MCMC chains were run from random trees for 1,000,000 generations and sampled every 100 generations with the burn-in value of 25%. The remaining trees were used to calculate PP values. All trees were visualized in FigTree v1.4.0 (Rambaut 2012) and the final layout was done with Microsoft PowerPoint. The final alignment and trees were registered in TreeBASE (2019) under the submission ID: 25248

## Phylogenetic conclusions

LSU sequence data of representative families comprised 39 strains, including two outgroup strains of *Parmularia styracis* Lév. (VIC42447, VIC42450). The alignment contained 32,916 characters (LSU: 1-844) including alignment gaps. The best scoring RAxML tree was selected to represent the relationships among taxa with a final likelihood value of -5964.8722. The matrix had distinct alignment patterns with 12.01% undetermined characters or gaps. Estimated base frequencies were:

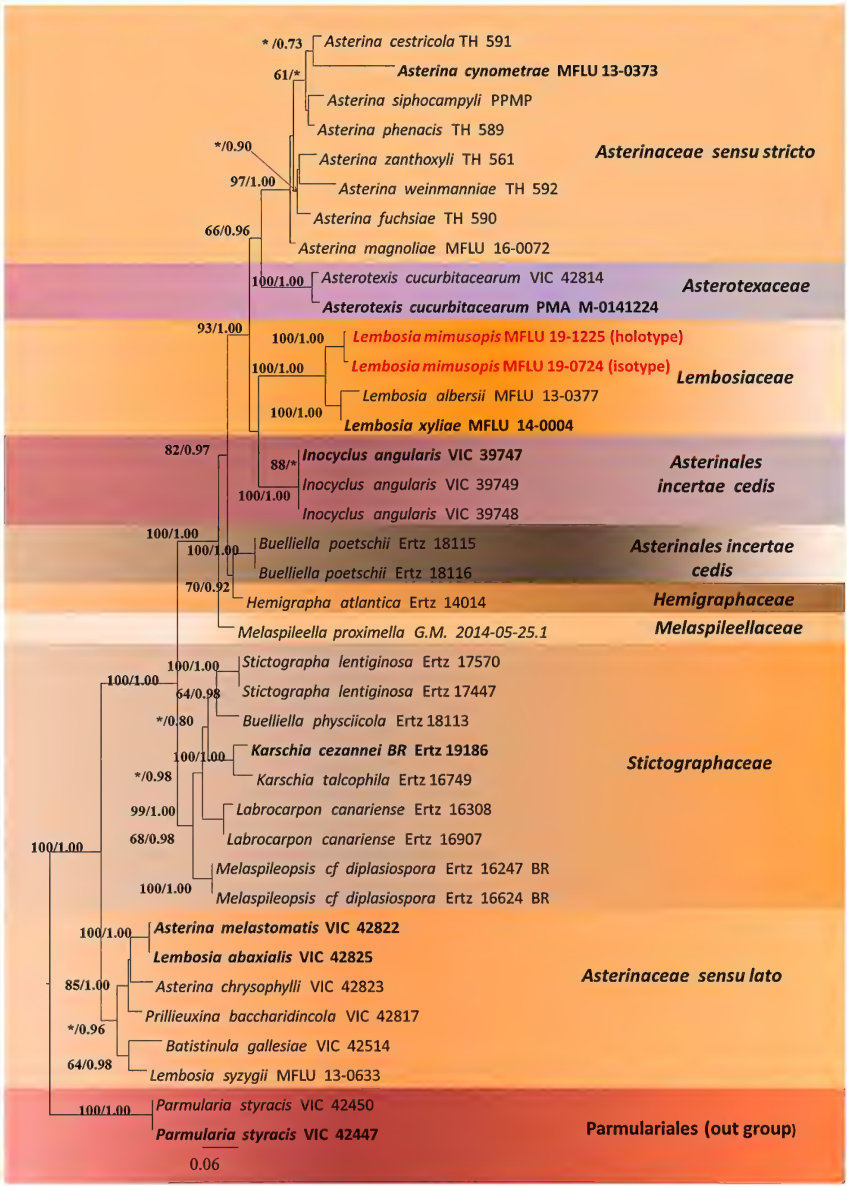


FIGURE 1. Phylogram of *Lembosia* and related genera, generated from maximum likelihood (RAxML) based on LSU sequence data. ML bootstrap support (≥60%) and Bayesian posterior probability (≥ 0.80) are indicated above the branches as ML/PP. The tree is rooted in *Parmularia styracis* (VIC 42447, VIC 42450). Ex-type strains are in bold and the newly generated sequences are in red.

A = 0.234839, C = 0.253073, G = 0.326282, T = 0.185806; substitution rates AC = 0.902460, AG = 2.658802, AT = 0.629941, CG = 1.176560, CT = 8.729825, GT = 1.000000; gamma distribution shape parameter α = 0.3196 (Fig. 1).

*Lembosia albersii, L. xyliae*, and our new taxon clustered with high statistical support (100% ML, 1 BYPP), forming a sister clade to three strains of *Inocyclus angularis* Guatim. & R.W. Barreto (VIC 39747, VIC 39748, VIC 39749). The new taxon is clearly distinct from the two other species in the clade with high statistical support. However, *L. abaxialis*, *L. brigadeirensis*, and *L. syzygii* clustered with species in *Asterina* sensu lato.

## Taxonomy

***Lembosia mimusopis*** Marasinghe, Daya., Maharachch., Hongsanan & K.D. Hyde, **sp. nov.** Fig 2

IF 556875

Differs from *Lembosia bomjardinensis* in having ascospores that are sheathed when immature.

Type: Thailand, Chiang Rai, MFU Botanical Gardens, on living leaves of *Mimusops elengi* L. (*Sapotaceae*), 19 December 2018, M.W.D. Sandamali BSM-2019a (**Holotype,** MFLU 19-1225; GenBank MN563123. **Isotype,** MFLU 19-0724; GenBank MN565982).

Etymology: referring to the host genus *Mimusops* from which the fungus was collected.

Colonies hypophyllous on *Mimusops elengi* leaves forming blackened circular to irregular areas, single to confluent. Hyphae 3.2–5 µm diam. ($\bar{x}$ = 4.5 µm, n = 10), superficial, brown, straight to undulate, branching opposite to irregular at acute to wide angles, reticulate. Appressoria 4–5.5 µm diam. ($\bar{x}$ = 4.5 µm, n = 10), unicellular, brown, irregular, opposite to alternate.

Sexual morph: Thyriothecia 350–500 × 350–450 µm ($\bar{x}$ = 451 × 393 µm, n = 6), scattered, sub-dense, initially rounded, elongated at maturity, longitudinally dehisced at the centre, margin crenate to fimbriate, borne on the surface of mycelium. Pseudoparaphyses not observed. Asci 55–65 × 45–55 µm ($\bar{x}$ = 60 × 48 µm, n = 10), 8-spored, bitunicate, sub-globose to globose, apical region of asci usually thick, opaque, ocular chamber not observed, not staining blue in IKI. Ascospores 25–30 × 8–11 µm ($\bar{x}$ = 28 × 9 µm, n = 10), overlapping 4–5-seriate, oblong to obovoid, with broad to narrowly rounded ends, lower cell slightly longer and narrower, hyaline, brown when mature, covered with mucilaginous sheath when immature, 1-septate, constricted at the septum, verruculose.

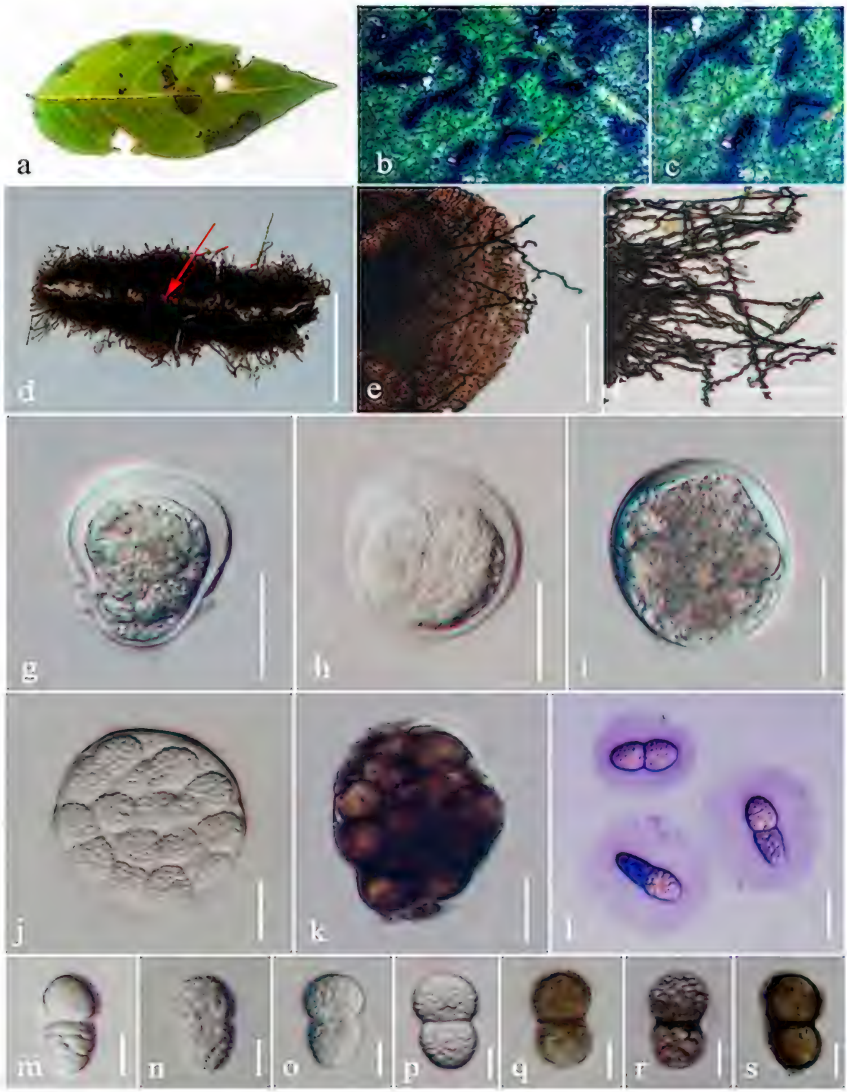


Figure 2. *Lembosia mimusopis* (holotype, MFLU 19-1225): a. Leaf sample; b, c. Thyriothecia on host substrate; d. Squash mount of thyriothecium showing longitudinal fissure (red arrow); e. Upper wall of thyriothecium; f. Hyphae with appressoria; g–j. Immature asci; k. Mature ascus; l. Ascospores with mucilaginous sheath stained with blue ink; m–s. Immature to mature ascospores. Scale bars: d–f = 100 µm; g–k = 20 µm; l–s = 10 µm.

Asexual morph: Undetermined.

Comments: *Lembosia mimusopis* (FoF06234) is morphologically similar to *L. bomjardinensis* (Bat.) Hongsanan & K.D. Hyde [≡ *Yamamotoa bomjardinensis* Bat.] in its globose to subglobose, apedicellate asci with a thick hyaline apical region and oblong to obovoid, 1-septate, hyaline to brown ascospores with a verruculose wall. However, *L. mimusopis* is distinguished by having sheathed ascospores when immature; and in its sapotaceous host in contrast to the proteaceous host of *L. bomjardinensis*. Attempts to isolate *L. mimusopis* onto potato dextrose agar and malt extract agar were unsuccessful so that the DNA was extracted directly from fruiting bodies.

## Discussion

*Lembosia mimusopis* represents the first record of a *Lembosia* species from *Mimusops* (*Sapotaceae*). However, *L. sapotacearum* Mibey was recorded from *Sapotaceae* in East Africa (Mibey & Hawksworth 1997). *Lembosia* species are also recorded from other host families (*Araucariaceae, Asparagaceae, Dipterocarpaceae, Ebenaceae, Lauraceae, Myrtaceae, Proteaceae, Orchidaceae;* Index Fungorum 2019). A comprehensive comparison of morphology within this genus is complicated by the lack of good illustrations and descriptions of previously recorded species. Host-specificity of *Lembosia* species has not been phylogenetically studied (Hongsanan & al. 2014). Hongsanan & al. (2014) synonymized *Trichamelia* Bat., Viegasia Bat., and *Yamamotoa* Bat. under *Lembosia*. Dai & al. (2018) pointed out the necessity of recollecting the type species, *L. tenella,* to obtain molecular data to establish its placement within *Asterinales*. In agreement with the results reported by Dai & al. (2018), our study also shows that four *Lembosia* species form a sister clade with *Inocyclus* in *Asterinales*.

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# *Fibroporia gossypium* isolated from an indoor environment in Argentina

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Abstract—Using culture studies and molecular techniques, the brown rot polypore *Fibroporia gossypium* was identified from a cellar in an old building of Buenos Aires city, the second record of the species in an urban environment in Argentina. The fungus formed large mycelial masses hanging from a wooden support and growing on the walls but did not form basidiomes. The conspecificity of specimens from distant regions is considered and the origin of the present finding is discussed.

Key words—multigene analysis, South America, taxonomy, wood-rotting fungus

## Introduction

*Fibroporia gossypium* [≡ *Antrodia gossypium*] is a widely distributed brown rot wood-decay basidiomycete occurring in coniferous stands in Europe (Spirin 2007, Bernicchia & al. 2012, Ryvarden & Melo 2014, Żółciak & al. 2016), North America (Lombard 1990), and Asia (Yuan & Dai 2008). It is

also found on *Nothofagus* spp. in Patagonia, Argentina (Rajchenberg 2006, Rajchenberg & al. 2011).

The species was originally described as *Poria gossypium* (holotype LPS 25495) from a specimen isolated from vine plant borders in La Plata, Buenos Aires Province, 122 years ago (Spegazzini 1898, Rajchenberg 1984, Rajchenberg & Wright 1987). After that, the species was not recorded in Argentina until studies in the southern region of the country (Rajchenberg 2006).

During summer of 2018, an extensive mycelium of a wood-decay basidiomycete was found in a basement located in the plant pathology laboratory of the School of Agriculture of the University of Buenos Aires. There was mycelium hanging from a door frame (Fig. 1A) and typical mycelial cords (rhizomorphs) were observed on the walls (Fig. 1B). The fungus was also covering the furniture (Fig. 1C) and had decayed a wooden door, made from an unidentified gymnosperm, extremely decayed, and found fallen to the floor (Fig. 1D). The basement had been formerly used as a cheese ripening chamber with an environment of high relative humidity, cool temperature, and darkness. More recently, the basement had been used as a depository for books and materials used in the dairy industry and had been abandoned for at least 15 years until the mycelium was found covering the walls, wooden shelves, door, and door-frame.

The aim of this work was to identify and to discuss the presence of *F. gossypium* growing in an indoor environment in Buenos Aires city, Argentina. This is the second urban record of the species, originally described by Spegazzini (1898) from La Plata city (ca. 60 km from Buenos Aires).

## Materials & methods

### Isolation and cultural studies

The fungus was isolated directly from the mycelium hanging from the door frame and cultured in 2% MEA, 2% PDA, yeast media and 2% water agar medium and incubated at both dark and natural light at 24 °C. The micro and morphological characteristics were observed regularly under the microscope.

The strain, named ARG_2018_Agro, was cultured on 1.25% MEA (Difco Lab.) in the dark at 25 °C (Nobles 1965), and macro- and micromorphological characters were recorded weekly for 6 weeks. The species was identified by means of keys based on mycelial characters (Nobles 1965, Stalpers 1978) and codified according to Nobles (1965). Oxidase reactions were performed using gallic (GAA) and tannic (TAA) acid agar media (Davidson & al. 1938) and tyrosine, p-cresol and guaiacol agar media (0.2%) according to Boidin (1954). The relative intensity of the reaction, recorded one week after incubation in the dark at 25 °C, was indicated with plus or minus signs.

Fig. 1. *Fibroporia gossypium* (ARG_2018_Agro), sampling site, with mycelium covering the walls, furniture, and wooden door frame. A. Conspicuous mycelial flaps hanging from the wooden frame; B, C. Mycelium and rhizomorphs covering furniture and walls; D. Wooden door rotted by the fungus.

Strain ARG_2018_Agro is conserved in the Department of Plant Pathology, School of Agriculture, University of Buenos Aires, Buenos Aires City (DPP FAUBA).

**Molecular studies**

The genomic DNA was extracted from 7-day-old fungal cultures grown in 2% MEA culture plates using the PuriPrep T Kit, according to the manufacturer's

instructions. Five genomic regions were included in the analyses, following Chen & al. (2017). DNA was amplified in 50 µl volumes containing 10 mM PCR buffer supplied by the manufacturer, 50 mM $MgCl_2$, 1 mM of each dNTP, 10 mM of each primer, 1 U Recombinant Taq DNA polymerase, and 5 µl fungal genomic DNA.

The internal transcribed spacer (ITS) region of the isolates was amplified using the universal primers ITS1 and ITS4 under the following PCR conditions: denaturation at 94 °C for 3 min, 50 cycles of denaturation at 94 °C for 45 s, annealing at 57 °C for 45 s, extension at 72 °C for 1 min and a final extension at 72 °C for 7 min (Schoch & al. 2012). The nLSU regions were amplified with primer pairs LR0R and LR7 (https://sites.duke.edu/vilgalyslab/rdna_primers_for_fungi/): the nSSU regions with the primer pair NS1 and NS4 (White & al. 1990); the mtSSU regions with the primer pair MS1 and MS2 (White & al. 1990); the tef1 with the primer pair EF1-983F and EF1-1567R (Rehner & Buckley 2005); and rpb2 with the primer pair bRPB2- 6F and bRPB2-7R (Matheny 2005). The PCR conditions for nSSU, mtSSU, tef1, and rpb2 are described in Chen & al. (2017). The PCR products were purified and sequenced at sequencing service of Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (Argentina) and at Macrogen Sequencing Service (Macrogen, Korea), with the same primers.

### Phylogenetic analysis

BLAST queries were performed in GenBank. As a framework for taxon selection, we used sequences of *Fibroporia* and its related genera in the antrodia clade obtained by Chen & al. (2017) (Table 1). Two data sets were analyzed for this study: concatenated analyses of the ITS, nLSU, nSSU, mtSSU, tef1, and rpb2 gene regions and the ITS region to ascertain the phylogenetic structure within the *F. gossypium–pseudorennyi* clade. Nucleotide sequences for the ITS, nLSU, nSSU, mtSSU, tef1, and rpb2 gene regions were initially edited using BIOEDIT 7.0.9.0 (Hall 1999) then aligned using L-INS-i strategy as implemented in MAFFT v 7.0 (Katoh & Standley 2013) and manually adjusted using MEGA version X (Kumar & al. 2018). The final ITS data set comprised 45 sequences and 668 characters including gaps, the nLSU data set comprised 45 sequences and 1319 characters including gaps, while the mtSSU, tef1, and rpb2 data sets comprised 40 each and 1015 (mtSSU), 487 (tef1), and 651 (rpb2) characters including gaps. The datasets were combined for concatenated analyses using Mesquite 3.40 (Maddison & Maddison 2018). ITS data set for the extended *Fibroporia* species analysis comprised 29 sequences and 623 characters including gaps. The best nucleotide substitution model was estimated by using the Akaike Information Criterion (AIC) (Akaike 1974) using JMODELTEST 2 2.1.1 (Guindon & Gascuel 2003, Darriba & al. 2012) and were GTR+I+G for ITS, nLSU, nSSU, and rpb2; GTR+G for mtSSU; and TIMef+I+G for tef1. Maximum likelihood (ML) phylogenetic trees for the combined data was estimated under these models in RAxML 7.2.8 (Stamatakis 2014), using the default parameters, executed on the CIPRES (Cyberinfrastructure for Phylogenetic Research) Science Gateway V.3.1 (http://www.phylo.org/sub_sections/portal/, Miller & al. 2010) with bootstrap support values (MLB) calculated with 1000 repetitions, and Bayesian

Table 1. Sequences of *Fibroporia* and related genera used in the molecular analyses.

| Taxon | Voucher | Origin | GenBank accession number | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | ITS | NLSU | NSSU | MTSSU | TEF1 | RPB2 | |
| *Amyloporia xantha* | Cui 11544 | China | KR605817 | KR605756 | KR605918 | KR606018 | KR610746 | KR610836 | Han & al. 2016 |
| *Antrodia heteromorpha* | Dai 12755 | USA | KP715306 | KP715322 | KR605908 | KR606009 | KP715336 | KR610828 | Chen & Cui 2015<br>Han & al. 2016 |
| *Antrodia serialis* | Cui 10519 | China | KP715307 | KP715323 | KR605911 | KR606011 | KP715337 | KR610830 | Han & al. 2016 |
| | Cui 9706 | China | KR605811 | KR605750 | KR605910 | KR606010 | KR610741 | KR610829 | Han & al. 2016 |
| *Antrodia serpens* | Dai 7465 | China | KR605813 | KR605752 | KR605913 | KR606013 | KR610742 | KR610832 | Han & al. 2016 |
| *Antrodia tanakae* | Cui 9743 | China | KR605814 | KR605753 | KR605914 | KR606014 | KR610743 | KR610833 | Han & al. 2016 |
| *Daedalea dickinsii* | Yuan 1090 | China | KR605790 | KR605729 | KR605878 | KR605981 | KR610711 | KR610802 | Han & al. 2016 |
| *Daedalea quercina* | Dai 12659 | Finland | KP171208 | KP171230 | KR605887 | KR605990 | KR610719 | KR610810 | Han & al. 2015; 2016 |
| *Fibroporia albicans* | Cui 9464 | China | KC456250 | KR605758 | KR605920 | KR606019 | KR610748 | KR610838 | Chen & al. 2015 |
| | Cui 9495 | China | KC456252 | KU550484 | KU550524 | KU550505 | KU550557 | KU550542 | Chen & al. 2015; 2017 |
| | Cui 9504 | China | KC456251 | KU550485 | KU55052 | KU550504 | KU550558 | KU550543 | Chen & al. 2017 |
| | Dai 10595 | China | KC456249 | KR605759 | KR605921 | KR606020 | KR610749 | KR610839 | Chen & al. 2015 |
| *Fibroporia bambusae* | Dai 16210 | China | KU550479 | KU550486 | KU550526 | KU550506 | KU550559 | KU550544 | Chen & al. 2017 |
| | Dai 16211 | China | KU550480 | KU550487 | KU550527 | KU550507 | KU550560 | KU550545 | Chen & al. 2017 |
| | Dai 16212 | China | KU550481 | KU550488 | KU550528 | KU550508 | KU550561 | KU550546 | Chen & al. 2017 |
| *Fibroporia bohemica* | BJFC 12750 | Czechia | KT895883 | KU550489 | KU550529 | KU550509 | KU550562 | KT895900 | Chen & al. 2017 |
| *Fibroporia ceracea* | Dai 13013 | China | KU550476 | KU550490 | KU550530 | KU550510 | KU550563 | KU550547 | Chen & al. 2017 |

| Taxon | Voucher | Origin | GenBank accession number | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | ITS | NLSU | NSSU | MTSSU | TEF1 | RPB2 | |
| | CLZhao 2308 | China | MH114651 | —— | —— | —— | —— | —— | Zhao [unpub.] |
| *Fibroporia citrina* | HUBO 7715 | Italy | GU991573 | —— | —— | —— | —— | —— | Bernicchia & al. 2012 |
| | Cui 11604 | China | KU550473 | KU550492 | KU550533 | KU550512 | KU550568 | KU550551 | Chen & al. 2017 |
| | Cui 9683 | China | KC456253 | KU550491 | KU550531 | KU550511 | KU550564 | KU550548 | Chen & al. 2017 |
| *Fibroporia gossypium* | HUBO 7724 | Italy | HM590880 | —— | —— | —— | —— | —— | Bernicchia & al. 2012 |
| | Cui 9472 | China | KU550474 | KU550494 | KU550534 | KU550514 | KU550567 | KU550550 | Chen & al. 2017 |
| | LY BR 3914 | France | KU550475 | KU550495 | —— | KU550515 | KU550569 | KU550552 | Chen & al. 2017 |
| | LY BR 3994 | Argentina | KT895885 | KU550496 | KU550535 | KU550516 | KU550570 | KT895902 | Chen & al. 2017 |
| | X1403 | Finland | KC595905 | —— | —— | —— | —— | —— | Ortiz-Santana & al. 2013 |
| | CIEFAP 131 | Argentina | JF713075 | —— | —— | —— | —— | —— | Rajchenberg & al. 2011 |
| | FG2011 | Poland | KF725876 | —— | —— | —— | —— | —— | Żółciak & al. 2016 |
| | ARG_2018 _Agro | Argentina | MT274740 | MT162434 | MT162453 | MT176423 | MT333221 | MT292789 | **This study** |
| *Fibroporia norrlandica* | 4115 | Finland | KC595907 | KC595907 | --- | --- | --- | --- | Han & al. 2014 |
| | 4122 | Finland | KC595908 | KC595908 | --- | --- | --- | --- | Ortiz-Santana & al. 2013 |
| | UC2022910 | USA | KP814248 | --- | --- | --- | --- | --- | Rosenthal & al. 2017 |
| *Fibroporia pseudorennyi* | X1377 | France | KC595927 | KC595927 | --- | --- | --- | --- | Ortiz-Santana & al. 2013 |
| | X1384 (holotype) | Russia | KC595928 | KC595928 | --- | --- | --- | --- | Ortiz-Santana & al. 2013 |

| Taxon | Voucher | Origin | GenBank accession number | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | ITS | NLSU | NSSU | MTSSU | TEF1 | RPB2 | |
| *Fibroporia radiculosa* | Cui 11404 | China | KP145011 | KR605760 | KR605922 | KR606021 | KR610750 | KR610840 | Chen & al. 2015, Han & al. 2016 |
| | Cui 2796 | China | KC456247 | KU550497 | KU550536 | KU550517 | KU550571 | KU550553 | Chen & al. 2017 |
| | Dai 13041 | China | KP145010 | KU550499 | KU550538 | KU550519 | KU550573 | KU550554 | Chen & al. 2017 |
| | Dai 6473 | China | FJ644283 | KU550498 | KU550537 | KU550518 | KU550572 | KT895899 | Chen & al. 2017 |
| *Fibroporia vaillantii* | FP 90877 R | USA | KC585345 | KC585170 | --- | --- | --- | --- | Ortiz-Santana & al. 2013 |
| | RWD 63 263 | USA | KC585346 | KC585172 | --- | --- | --- | --- | Ortiz-Santana & al. 2013 |
| *Fomitopsis pinicola* | Cui 10312 | China | KR605781 | KR605720 | KR605856 | KR605960 | KR610689 | KR610780 | Han & al. 2016 |
| *Laetiporus sulphureus* | Dai 12154 | China | KF951295 | KF951302 | KR605924 | KR606023 | KR610752 | KR610841 | Song & al. 2014, Han & al. 2016 |
| | Dai 12826 | China | KR605819 | KR605762 | KR605925 | KR606024 | KR610753 | KR610842 | Han & al. 2016 |
| *Oligoporus lacteus* | Cui 12206 | China | KR605820 | KR605763 | KR605926 | KR606025 | KR610754 | KR610843 | Han & al. 2016 |
| *Piptoporus betulinus* | Cui 10756 | China | KR605797 | KR605736 | KR605894 | KR605997 | KR610725 | KR610815 | Han & al. 2016 |
| *Piptoporus soloniensis* | Cui 11390 | China | KR605803 | KR605742 | KR605901 | KR606003 | KR610733 | KR610822 | Han & al. 2016 |
| | Dai 11872 | China | KR605804 | KR605743 | KR605902 | KR606004 | KR610731 | KR610823 | Han & al. 2016 |
| *Postia duplicata* | Cui 10366 | China | KF699124 | KJ684975 | KR605927 | KR606026 | KR610755 | KR610844 | Shen & al. 2015, Han & al. 2016 |
| *Pseudofibroporia citrinella* | He20120721-15 | China | KU550477 | KU550500 | KU550539 | KU550520 | KU550574 | KU550555 | Chen & al. 2017 |
| | Yuan 6181 | China | KU550478 | KU550501 | KU550540 | KU550521 | KU550575 | KU550556 | Chen & al. 2017 |
| *Trametes polyzona* | Cui 11040 | China | KR605824 | KR605767 | KR605932 | KR606029 | KR610760 | KR61084 | Han & al. 2016 |
| *Trametes suaveolens* | Cui 11586 | China | KR605823 | KR605766 | KR605931 | KR606028 | KR610759 | KR610848 | Han & al. 2016 |

inferences (BI) of phylogenies in MrBayes v.2.2 (Ronquist & al. 2012). Four independent chains were run for 8 million generations; trees were sampled every 1000 generations. Log files for each run were viewed in Tracer v1.6.0 (http://evolve.zoo.ox.ac.uk/software.html/tracer/) to determine convergence. Trees generated prior to stationarity were discarded, and the remainder of the trees was summarized in a majority rule consensus tree from the four independent runs. Branch support was assessed using posterior probabilities (BPP) calculated from the posterior set of trees after stationarity was reached. Sequences of *Trametes suaveolens* (L.) Fr. (Cui 11586) and *Trametes polyzona* (Pers.) Justo (Cui 1104042) were used as outgroup in the concatenated analyses of the ITS, nLSU, nSSU, mtSSU, tef1 and rpb2 gene regions and sequences of *Pseudofibroporia citronella* Yuan Y. Chen & B.K. Cui (He 20120721-15 and Yuan 6181) were used as outgroup in the ITS region analyses. The final alignments were deposited in TreeBASE (http://www.treebase.org), under accession ID25872.

## Taxonomy

***Fibroporia gossypium*** (Speg.) Parmasto,
Consp. System. Corticiac.: 207. 1968. FIGS 1, 2

≡ *Poria gossypium* Speg., Anales Mus. Nac. Buenos Aires 6: 169. 1898.

≡ *Antrodia gossypium* (Speg.) Ryvarden, Norweg. J. Bot. 20: 8. 1973.

Nobles culture code: 1.3.7.34.35.36.38.43.51.55.

GROWTH CHARACTERISTICS: Mats white, cottony, loose at first, appressed during the second week, powdery in the following weeks; margins distinct, even, or finely fimbriate; odorless; reverse unchanged; the colony reaching a radius of 17 mm after 7 da on 1.25% MEA, covering the plate in three weeks; oxidase reactions negative, GAA at 7 da 25–27 mm in diam, no growth on TAA.

HYPHAL CHARACTERISTICS: all hyphae staining in phloxine, thin-walled, mostly clamped with single clamps, rarely simple-septate, (2.5–)3.5–5(–6) µm diam. Crystals present from the first week, 3–4 × 2–3 µm. From the second week terminal chlamydospores 5–7(–11) × 4–6 µm and arthrospores (6–)9–15(–28) × 2–4 µm, very abundant at the sixth week.

SPECIMEN EXAMINED: **ARGENTINA, BUENOS AIRES CITY, University of Buenos Aires**, School of Agriculture, 34°35′30″S 58°28′46″W, basement wood-decay, December 2018, F. J. Sautua (DPP FAUBA ARG_2018_Agro).

COMMENTS: The description does not completely agree with Lombard (1990; as *Antrodia "gossypia"*) and the southern Argentina specimens studied by Rajchenberg (2006), because those strains showed few clamped hyphae, there was no mention of the presence of chlamydospores or arthrospores,

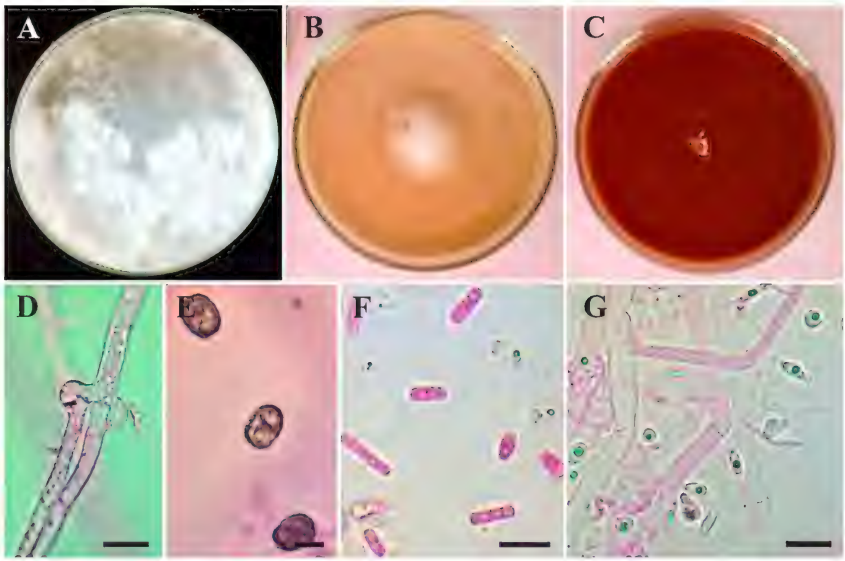


Fig. 2. *Fibroporia gossypium* (ARG_2018_Agro), cultural characteristics. A. Six-week-old culture; B. GAA medium; C. TAA medium; D. Clamp connections; E. Crystals; F. Arthrospores; G. Chlamydospores. Scale bars = 10 µm.

and growth was very slow, covering the plates after only after 7 weeks of incubation. However, Spirin (2007) noted that one specimen of *F. gossypium* (LE 210088) had thick-walled chlamydospores in the subiculum.

After a four-month incubation in 2% MEA, 2% PDA, yeast media, and 2% water agar medium, whether cultured in the dark or in natural light, there was no production of basidiomes.

### Phylogeny

Phylogenetic analyses based on the ITS and the combined concatenated data set ITS + nLSU + nSSU + mtSSU + tef1 + rpb2 for the antrodia clade (Figs. 3, 4) clustered strain ARG_2018_Agro and two Argentinian specimens [CIEFAP 131 from Chubut and CIEFAP M. Rajchenberg 11443 from Tierra del Fuego (Ushuaia, Tierra Mayor, in pure *Nothofagus pumilio* forests, on fallen trunk, 22 March 1998; duplicate as LY-BR 3994, cf. Chen & al. 2017] within the monophyletic group *F. gossypium* that includes *F. gossypium* and *F. pseudorennyi* (BPP = 1.0, MLB = 0.99, Fig. 3; BPP = 1.0, MLB = 0.95, Fig. 4).

## Discussion

Molecular analysis led to the identification of the ARG_2018_Agro strain found in the humid basement as the decay basidiomycete *Fibroporia gossypium*. The cultural characters showed, on the other hand, differences from previous descriptions of this species, in particular an absence of chlamydospores or arthrospores, fewer clamped hyphae, and slower growth (Lombard 1990, Rajchenberg 2006). Cultural features have facilitated separation and identification of different basidiomycete groups (Nobles 1965, Stalpers 1978); however, conflicting characters have also been noticed in such cultural studies.

Strain ARG_2018_Agro produced a brown rot decay, as indicated by the substrate from which it was isolated and the tannic acid agar media results. *Fibroporia gossypium* has already been associated with gymnosperms in the Northern Hemisphere and on *Nothofagus* sp. in the Patagonian Andes forests of Argentina (Rajchenberg 2006).

Spegazzini described the original material of *Poria gossypium* from border plants of (cultivated) vines near La Plata city: "Hab. In ollis vinariis ad plantas colendas adhibitis, in La Plata, aest. 1890 (leg. Cl. Fl. Ameghino)" (Spegazzini 1898), although he wrote on holotype envelope: "In limis plantarum, Fl. Ameghino, Aest. 1890" (Rajchenberg & Wright 1987). This exotic environment and host present the unusual situation of a taxon that was first described from a strange habit while it was naturally growing in other situations. While the species was widely recorded in the Northern Hemisphere (e.g., Lowe 1966, Núñez & Ryvarden 2001, Dai & Niemelä 2002, Ryvarden & Melo 2014), it was only recorded from the Southern Hemisphere from Patagonia, Argentina, many years later, there growing exclusively on fallen trunks and branches of different *Nothofagus* spp. (Rajchenberg 2006), contrasting with other geographical areas where it is mainly recorded on coniferous wood. The taxon is unknown from other austral continents.

How did this taxon arrive and profusely develop its mycelium in a cellar-like, indoor environment? We have no answer to that. An exotic origin is a possible explanation. Certainly, its dissemination and arrival seem to be an anomaly, as the species is not present as such in Buenos Aires city and is not associated with the introduced tree species planted in the city's groves (Robles & al. 2011, 2015). Its establishment and profuse growth has most possibly been encouraged by the still environment and optimum temperatures present in the abandoned room where it developed.

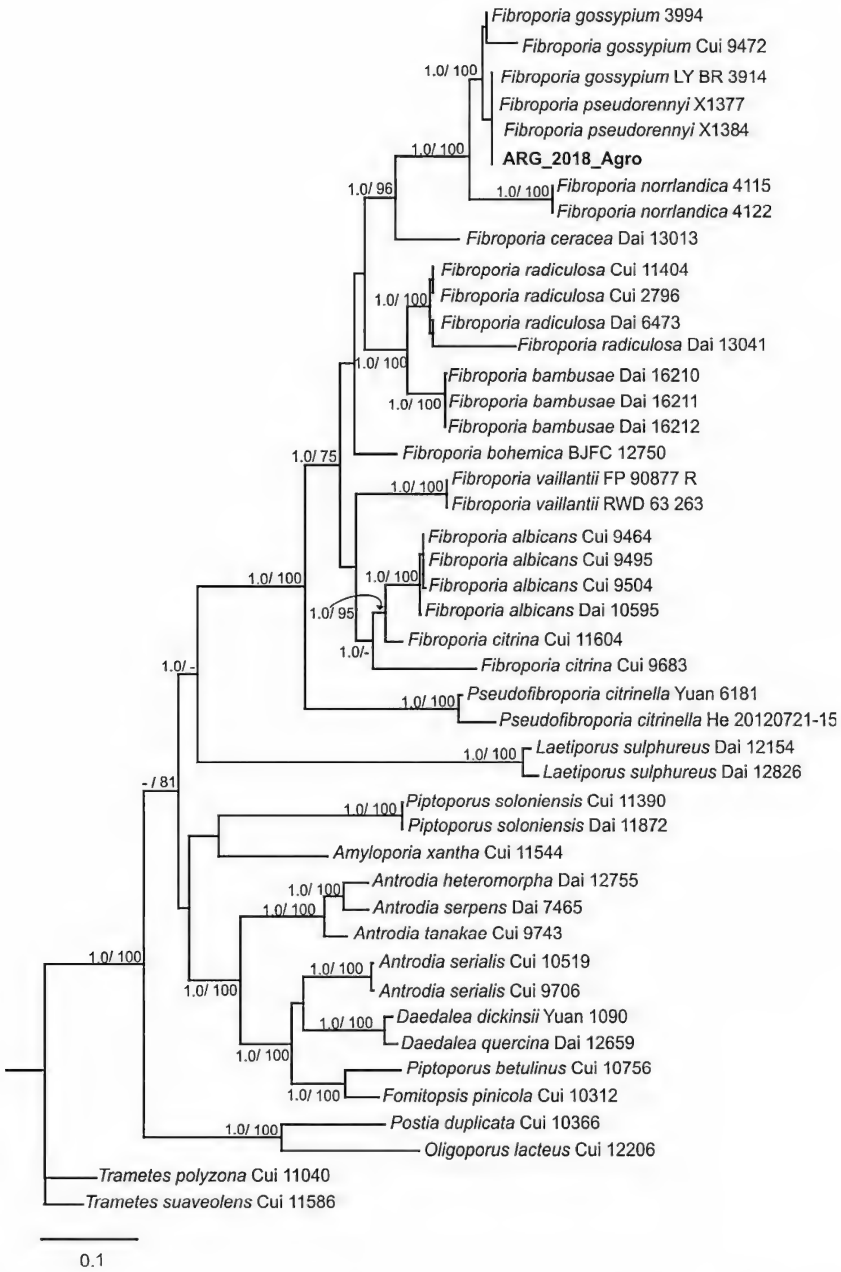


FIG. 3. ML tree based on the combined concatenated data of ITS + nLSU + nSSU + mtSSU + tef1 + rpb2 including 45 sequences. BPP >0.95 and MLB >50% are marked above the nodes.

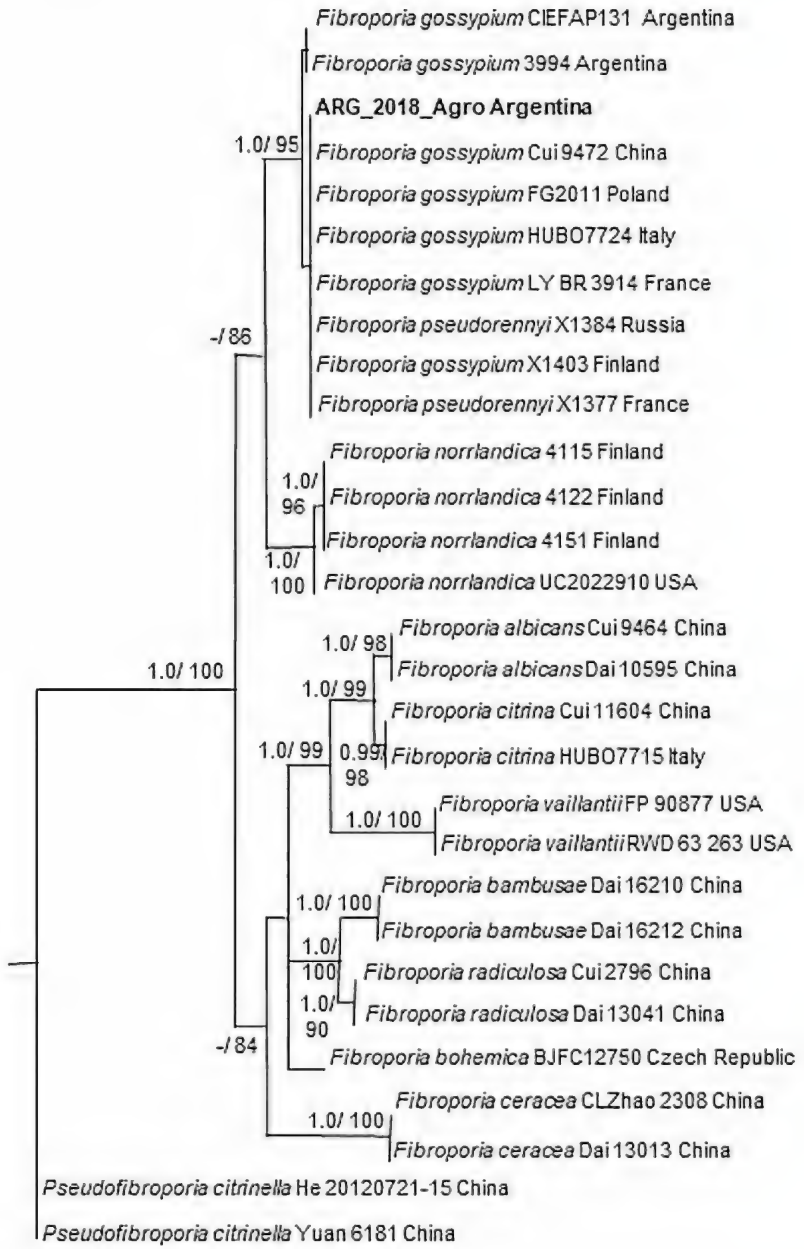


FIG. 4. The 50% majority-rule consensus tree from Bayesian inference of the ITS region including 29 sequences. BPP >0.95 and MLB >75% are marked above the nodes.

*Leptoporus destructor* var. *resupinatus* Bourdot & Galzin (1928: 547) was accepted as a form of *Fibroporia gossypium* by Lowe (1966, as *Poria gossypium*), Parmasto (1968, as *F. gossypium*), Domański (1972, as *F. gossypium*) and Ryvarden (1976, as *Antrodia gossypium*), growing on mine poles where environmental conditions are constant. The type species of *Fibroporia*, *F. vaillantii* (DC.) Parmasto, another "mine" fungus, is also associated with mine timber and affected wood in deteriorated buildings (Wainwright 1999, Cojocariu & al. 2007).

Spirin described *Fibroporia pseudorennyi* (Spirin) Spirin [≡ *Oligoporus pseudorennyi* Spirin] as phylogenetically close to *F. gossypium* but with smaller spores and lacking cystidia (Spirin 2004, 2007). *Fibroporia pseudorennyi* also produced chlamydospores ((6–)8–10 × 6–7.5 µm) in the basidiome margin; currently there are no cultural descriptions of *F. pseudorennyi*. Given the phylogenetic results of the present study and those of Chen & al. (2017) based on five genomic regions, the synonymy of *F. gossypium* and *F. pseudorennyi* is maintained (cf. Ryvarden & Melo 2014).

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# *Rubellofomes cystidiatus* and *Spongiporus floriformis* newly recorded for India

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Abstract-In an effort to document the macrofungi of Kerala state, India, we collected and identified two brown rot polypores, *Rubellofomes cystidiatus* and *Spongiporus floriformis*, which represent new records for India. Here we present taxonomic accounts of the two species.

Key words-biodiversity, brown rot, *Dacryobolaceae*, *Fomitopsidaceae*, *Polyporales*

## Introduction

*Rubellofomes* B.K. Cui & al. (*Fomitopsidaceae*, *Polyporales*) was erected to accommodate two brown rot polypores, *Fomitopsis cystidiata* B.K. Cui & M.L. Han and *F. minutispora* Rajchenb. According to Han & al. (2016), these two species were distinct from other *Fomitopsis* species by their purple pink to pinkish brown context. *Rubellofomes* can be distinguished from the closely related *Niveoporofomes* B.K. Cui & al. by its orange brown to dark brown pileal surface, purple pink to light pinkish brown context, and cylindrical to ellipsoid basidiospores. *Niveoporofomes* species produce ivory white to pale ochraceous pileus surfaces, white to ochraceous context, and ovoid to broadly ellipsoid basidiospores (Han & al. 2016). *Ungulidaedalea* B.K. Cui & al., another closely related genus, is separated by its ungulate, fragile fruit bodies and septate skeletal hyphae (Han & al. 2016). *Rubellofomes* has been reported previously from Argentina and China.

*Spongiporus* Murrill (*Dacryobolaceae*, *Polyporales*) was established by Murrill (1905), with *S. leucospongia* (Cooke & Harkn.) Murrill as the type

species. The genus accommodates brown rot species with a monomitic hyphal system (David 1980). *Spongiporus* representatives are characterized by an annual growth habit, effused reflexed to pileate basidiomata, a monomitic hyphal system with clamped generative hyphae, and hyaline, smooth, thin-walled, acyanophilous, inamyloid, allantoid to cylindrical basidiospores (Shen & al. 2019). *Spongiporus* is distributed in Asia, Europe, and North America (Murrill 1907, Ryvarden & Gilbertson 1994, Spirin & al. 2006). *Spongiporus* has been considered as a synonym of other two brown rot polypore genera, *Oligoporus* Bref. and *Postia* Fr., by different workers (Gilbertson & Ryvarden 1985, Ryvarden & Gilbertson 1994, Pildain & Rajchenberg 2013, Ryvarden & Melo 2014). According to Shen & al. (2019), *Oligoporus* basidiocarps are resupinate while *Spongiporus* produces effused reflexed to pileate basidiocarps. *Postia* species differ from *Spongiporus* by a universally solitary habit and narrower basidiospores (Shen & al. 2019). Pildain & Rajchenberg (2013) treat *Oligoporus* as a synonym of *Postia*. *Postia* species produce thin walled basidiospores and generative hyphae that are metachromatic in cresyl blue. *Oligoporus* representatives produce thick-walled cyanophilous spores and their hyphae are non-metachromatic in cresyl blue (Erkkilä & Niemelä 1986, Renvall 1992, Pildain & Rajchenberg 2013). Recent phylogenetic studies treat *Oligoporus*, *Postia*, and *Spongiporus* as independent genera (Ortiz-Santana & al. 2013, Binder & al. 2013, Cui & al. 2014, Shen & al. 2019). Seven species have been recognized in *Spongiporus* (He & al. 2019; www.indexfungorum.org).

Kerala state in India has a rich mycobiota, with a documented diversity of 148 polyporoid species (Adarsh & al. 2018). As part of studies on the polyporoid fungi of the region, two interesting species were encountered that were later found to represent new records for India. Here we present the taxonomic details of the two new polypore records.

## Materials and methods

Fruitbodies were collected from forest areas of the Kerala region of Western Ghats in India during the monsoon seasons. Macroscopic characters of fresh specimens were recorded. Microscopic observations were made on materials stained using aqueous solutions of 3% phloxine and 1% congo red and mounted in 5% aqueous KOH. Reaction of the basidiospores on treatment with Melzer's reagent and cotton blue was noted. Twenty basidiospores from each specimen were measured for obtaining the spore dimensions, range of spore quotient (Q, length/width ratio), and its mean value ($Q_m$). All collections examined are deposited at the Zamorin's Guruvayurappan College Herbarium, Kozhikode, Kerala, India (ZGC).

## Taxonomy

***Rubellofomes cystidiatus*** (B.K. Cui & M.L. Han) B.K. Cui, M.L. Han & Y.C. Dai, Fungal Diversity 80: 366. 2016 FIG. 1A–C

BASIDIOMATA annual, medium sized, pileate, hard, woody, light weight, when dry. PILEUS 30–65 mm long, 14–20 mm thick, sessile, broadly attached, difficult to detach from the host, applanate to slightly irregular with minute ridges and grooves, almost semicircular, glabrous, azonate, purplish brown to cinnamon brown (browner on drying), turning brownish black in KOH. MARGIN even, round, sterile, orange yellow. HYMENOPHORE poroid, pale purplish brown, more brownish on drying. PORES large, 1–3 mm wide, narrow towards margin, angular, pore tubes 10–15 mm long, concolorous with the hymenophore. CONTEXT 3–5 mm thick, very pale pinkish brown, homogeneous. ODOUR not distinctive. SPORE PRINT not observed.

HYPHAL SYSTEM dimitic, generative hyphae septate, with clamp connections, slightly thick to thick walled, skeletal hyphae unbranched to slightly branched. PILEIPELLIS an agglutinated cutis, easily crushed in sections, pileipellis hyphae 2–3 µm wide, slightly thick walled, yellowish brown. PILEAL TRAMA interwoven, generative hyphae 2–3 µm wide, septate, hyaline, mostly thick walled, branched, clamp connections present. HYMENIAL TRAMA interwoven, generative hyphae 2–3 µm wide, septate, hyaline, slightly thick to thick walled, branched, clamp connections present and skeletal hyphae 2–5 µm wide, hyaline, simple to rarely branched, aseptate, thick walled (1 µm). Skeleto-ligative hyphae not observed. CYSTIDIA 31–42 × 4–6 µm, clavate to cylindrical, hyaline, thick walled (1 µm), encrusted. BASIDIA 26–32 × 3–4 µm, clavate, 2-sterigmate, with basal clamp connections. BASIDIOSPORES 5–8 × 2–3 µm, Q = 3–4, $Q_m$ = 3.4, cylindrical, often slightly bent, smooth, thin walled, eguttulate, inamyloid in Melzer's reagent.

SPECIMEN EXAMINED: **INDIA. KERALA: Palakkad**, Silent Valley National Park, 11.0641°N 76.5378°E, on dead wood of unidentified tree, 14 January 2018, Vinjusha N. (ZGC VN667).

KNOWN DISTRIBUTION: China

COMMENTS: The present collection possesses slightly longer spores when compared with the original description of the material of *R. cystidiatus* ((4.8–)4.9–6.2(–6.5) × 2.0–2.8(–2.9) µm, Han & al. 2014). The pileus surface of *R. cystidiatus* was described as concentrically zonate (Han & al. 2014), but proper zonations or concentric rings were not observed in our collection. According to Han & al. (2014), the pore surface of *R. cystidiatus* is pale purple pink in fresh condition and turns clay buff to cinnamon brown on

drying. However, a pinkish tint was absent in our collection and its more purplish to brownish shades may be because the specimen was collected in a more or less dried form.

***Spongiporus floriformis*** (Quél.) Zmitr.,
Folia Cryptogam. Petropolitana 6: 90. 2018. FIG. 1D, E

BASIDIOMATA annual, small to medium, pileate, in clusters, slightly coriaceous and spongy when fresh, becoming hard on drying. PILEUS 15–30 mm long, 3–6 mm thick, laterally attached with a distinct region of attachment, rarely sessile, conchate, mostly flabelliform, sometimes semicircular, fruit bodies fused at the region of attachment, minutely pubescent to glabrous, cream white with pale brown zones when fresh, more brownish when mature, surface with concentric zones. MARGIN even to slightly wavy, incurved on drying, acute, sterile, concolor with the surface pileus. HYMENOPHORE poroid, creamish white, pale brown on bruising. PORES 6–8 per mm, absent near margin, angular to irregular, pore tubes 2–3 mm long, non-stratified, white when fresh, cream on bruising. CONTEXT 1–2 mm thick, white, homogenous. ODOR not distinctive. SPORE PRINT not observed.

HYPHAL SYSTEM monomitic, generative hyphae with clamp connections, thin to thick walled. PILEIPELLIS an irregular cutis, with scattered short erect agglutinated hyphal patches that are hyaline that are up to 20 µm long. PILEAL TRAMA interwoven, generative hyphae 2–5 µm wide, hyaline, branched, thin to mostly thick walled (1–2 µm), clamp connections frequent. HYMENIAL TRAMA interwoven, generative hyphae 2–4 µm wide, hyaline, branched, thin to mostly thick walled (1–2 µm), clamp connections frequent. CYSTIDIA and other sterile structures absent. BASIDIA 12–30 × 4–5 µm, clavate, 4-sterigmate, with basal clamp connections. BASIDIOSPORES 3–4 × 2–2.5 µm, Q = 1.5–1.6, $Q_m$ = 1.83, subcylindric to cylindric, hyaline, guttulate, usually with one or two large guttules, thin walled, smooth, inamyloid in Melzer's reagent.

SPECIMEN EXAMINED: **INDIA. KERALA: Kottayam**, Vagamon, Pine valley, 9.6862°N 76.9052°E, on rotten wood of *Pinus* species, 25 October 2015, Vinjusha N. (ZGC VN258).

KNOWN DISTRIBUTION: Asia, northern to southern Europe, circumpolar in the boreal conifer zone.

COMMENTS: The Indian specimen resembles *Postia tephroleuca* (Fr.) Jülich, a species already reported from India (Ranadive 2013). However,

FIG. 1: *Rubellofomes cystidiatus* (ZGC VN667): A. Basidiomata; B. Encrusted cystidia; C. basidiospore. *Spongiporus floriformis* (ZGC VN258): D. Basidiomata; E. Basidia with attached basidiospores. Scale bars: A = 25 mm, B = 15 µm, C = 10 µm, D = 10 mm, E = 6 µm.

*P. tephroleuca* fruitbodies are always sessile whereas fruit bodies of our collection have a distinct point of attachment. Also, *P. tephroleuca* basidiospores are longer and narrower (4.5–6 × 1–1.5 µm) than those of our collection (3–4 × 2–2.5 µm). According to the description from the isotype by Lowe (1975), *S. floriformis* produces conchate to often spathulate pileus with a central stem-like point of attachment. While Ryvarden & Melo (2014) describe the same collection as petal-like, sessile, or effused reflexed. According to Lowe (1975), the fruitbody trama contains some gloeoplerous hyphae; however, we did not observe any hyphae with gloeoplerous contents.

## Discussion

*Rubellofomes* comprises two species, *R. cystidiatus* and *R. minutisporus* (Rajchenb.) B.K. Cui & al. The presence of cystidia and larger spores distinguish *R. cystidiatus* from *R. minutisporus*. Except for the absence of distinct concentric zonations on the pileus and small variations in the pore surface colour and size of basidiospores, characters of our collection fit well with those in the original description of *R. cystidiatus* from South China (Han & al. 2014, as *Fomitopsis cystidiata*). Since then, the species has never been reported from elsewhere, and this is the first report of the species outside its type locality.

The Kerala collection of *Spongiporus floriformis* resembles *Postia tephroleuca*, except that *P. tephroleuca* has sessile fruit bodies and longer and narrower basidiospores. Possession of leptocystidia and amyloid tramal hyphae help separate *S. rhodophilus* Spirin & Zmitr. from our *Spongiporus* collection. Spirin & al. (2006) reported conidial cells on the pileus of *S. floriformis,* but we did not find any conidia in our collections. Reported hosts of *S. floriformis* are *Abies*, *Cedrus*, *Crataegus*, *Cupressus*, *Larix*, *Picea*, and *Pinus* (Ryvarden & Melo 2014). The collection from Kerala was obtained from dead wood of *Pinus*.

*Rubellofomes cystidiatus* and *Spongiporus floriformis* are new records for the mycobiota in India.

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# *Diplodia bulgarica*, a new record for Turkey

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Abstract—*Diplodia bulgarica* on *Malus domestica*, a new species for the Turkish mycobiota, is described briefly and illustrated. A pathogenicity test showed that *D. bulgarica* was highly aggressive to apple plants.

Key words—canker, *Botryosphaeriaceae, Dothideomycetes*

## Introduction

Apple (*Malus domestica* Borkh.) is an economically important horticultural crop in Turkey; more than 3,500,000 t of apples are produced in Turkey every year, making the country the fourth largest producer worldwide (FAO 2018). Fungal pathogens are major biotic stresses affecting apple tree growth and fruit production. *Botryosphaeriaceae* encompasses a range of morphologically diverse fungi that are either pathogens, endophytes, or saprophytes, occurring mainly on woody plants such as apple (Slippers & Wingfield 2007; Phillips & al. 2012, 2013). Among the 23 genera recognized in this family is *Diplodia* (Dissanayake & al. 2016). *Diplodia* is a large genus currently comprising more than 1000 species (Phillips & al. 2012). Species of *Diplodia* can cause canker, dieback, gummosis, fruit rot, and twig blight diseases of numerous woody hosts (Crous & al. 2006, Slippers & Wingfield 2007, Lazzizera & al. 2008, Phillips & al. 2012, Abdollahzadeh 2015, Nabi & al. 2020, Hinrichs-Berger & al. 2021). Recently, a botryosphaeriaceous species associated with canker symptoms of apple trees was observed in Eğirdir district of Isparta province, Turkey. The present paper reports the etiology of the disease.

## Materials & methods

In 2019, extensive canker symptoms were observed on apple trees in Eğirdir district of Isparta province, Turkey. Samples were collected from symptomatic trees showing cankers in branches and transported in a cooler to the laboratory. Fungi were isolated from canker margins after surface sterilization of samples in 2% sodium hypochlorite solution and rinsing in two changes of sterile distilled water and transferred to Petri plates (9 cm diam.) containing Potato Dextrose Agar (PDA). Plates were incubated 5–7 days at 25 °C, and the fungal isolate cultures were subculture using hyphal tips cut from the colonies under a dissecting microscope and placed, one colony per plate, onto PDA. Identification of isolates to species level was based on colony morphology and conidial characteristics, as described by Phillips & al. (2012). Isolates are maintained at the Department of Agricultural Biotechnology, Faculty of Agriculture, Aydın Adnan Menderes University, Aydın, Turkey (ADUAB).

The identity of isolates was confirmed by analysis of the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA). Genomic DNA was extracted from the mycelium from the surface of the PDA cultures using an Omega Bio-Tek SP-Fungal DNA Kit according to the manufacturer's instructions. After DNA extraction of two isolates (CEE-22, CEE-211), the rDNA ITS region was amplified using primers ITS1/ITS4 (White & al. 1990). Amplicons were purified and sequenced by RefGen (Ankara, Turkey). The nucleotide sequence was compared with the GenBank nucleotide collection database through nucleotide BLAST search.

Preliminary pathogenicity tests were carried out on potted 2-year-old apple seedlings (cv. Scarlet Spur) using the representative isolates from cankers; the most virulent isolate (CEE-22) was used for greenhouse test. The outer bark at the inoculation area was disinfected by spraying with 70% ethanol, and the plants were wounded with a scalpel through the full thickness of the bark along a 1-cm long slit and inoculated with a 5 mm plug from a 7-day-old culture grown on PDA and covered with parafilm. Control seedlings were similarly wounded and plugs of sterile PDA applied. The bark of the inoculated area was removed, and the lesion lengths recorded after four weeks. Re-isolation was done from the margins of infected tissues as described above and re-isolated fungi were identified by comparing colony and conidial morphological characteristics to the original inoculated isolate (CEE-22).

## Results

A total of 12 isolates were obtained by isolation from 12 cankered branches of apple trees collected from different localities of Eğirdir district of Isparta province, Turkey. Based on morphological and cultural characteristics, these isolates were tentatively identified as *Diplodia bulgarica*. The identity of two representative isolates (CEE-22 and CEE-211) was confirmed by DNA sequencing of the rDNA ITS region (GenBank accession no. MT239082 and MZ442352). The description and illustrations of *D. bulgarica* given below

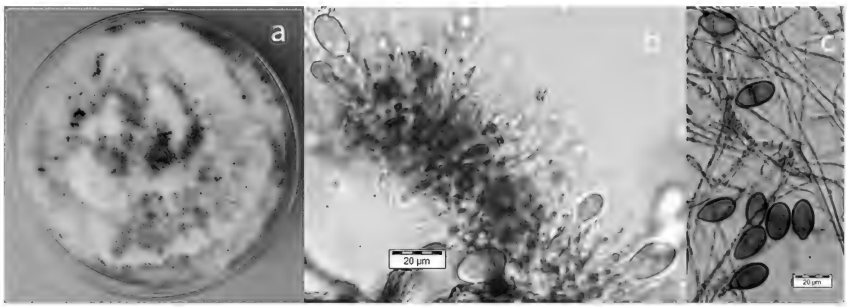


Fig. 1. *Diplodia bulgarica* (ADUAB – CEE-22): a. Colony on PDA; b. Conidia developing on conidiogenous cells; c. Septate and aseptate conidia.

are based on the Turkish collections of the material. This is the first report of *D. bulgarica* from Turkey.

## Taxonomy

***Diplodia bulgarica*** A.J.L. Phillips, J. Lopes & S.G. Bobev, Persoonia 29: 33. 2012. Fig. 1

Colony growth at 25 °C on PDA reaches 47 mm in diameter after 3 days. The colony was at first white, turned to gray to dark gray with irregular margin having dark centre and formed pycnidia after 20–25 days were immersed in culture. Pycnidia were globose to ovoid, dark brown to black. Conidia were aseptate, smooth, thick walled, oblong to ovoid, both ends broadly rounded, 19.8–29.0 × 10.4–15.1 µm, initially hyaline and later becoming pale brown and sometimes 1-septate at maturity.

Specimens examined: **TURKEY, Isparta Province, Eğirdir district,** Serpil village, from cankered branches of *Malus domestica*, 2 Nov. 2019, C. Eken CEE-22 (ADUAB; GenBank MT239082); 9 Nov. 2019, CEE-46 (ADUAB); Tepeli village, from cankered branches of *Malus domestica*, 8 Jan. 2019, C. Eken CEE-211 (ADUAB; GenBank MZ442352); 27 May 2019, CEE-212, CEE-223 (ADUAB); Balkırı village from cankered branches of *Malus domestica*, 7 Dec. 2019, C. Eken CEE-141 (ADUAB); Barla village from cankered branches of *Malus domestica*, 27 July 2019, C. Eken CEE-178, CEE-179 (ADUAB); Mahmatlar village from cankered branches of *Malus domestica*, 5 Oct. 2019, C. Eken CEE-186, CEE-191 (ADUAB); Sevinçbey village from cankered branches of *Malus domestica*, 24 Aug. 2019, C. Eken CEE-123, CEE-124 (ADUAB).

Symptoms of canker were observed on the stem of the inoculated apple seedlings. Canker lesions expanded to 52 ± 0.4 mm four weeks after inoculation and extended both upwards and downwards from the inoculation

sites. The pathogen was successfully re-isolated from all inoculated stems and isolate identity was confirmed based on colony and conidial morphological characteristics. In control treatments, no symptoms developed.

## Discussion

*Diplodia bulgarica*, originally isolated from dead twigs of *Malus sylvestris* in 2005, was described by Phillips & al. (2012). In our study, *Diplodia bulgarica* was isolated from apple trees (*M. domestica*) in the Eğirdir district of Isparta province of Turkey, showing canker symptoms consistent with previous reports (Abdollahzadeh 2015, Hanifeh & al. 2017, Bari & al. 2019, Nabi & al. 2020, Hinrichs-Berger & al. 2021).

The size of conidia of the Turkish isolates is within the ranges observed by Phillips & al. (2012; 22.5–28.0 × 14.5–18.5 µm), Hanifeh & al. (2017; 19–29 × 12.5–17.5 µm), and Nabi & al. (2020; 20.0–28.0 × 9.0–14.5 µm). The identity of isolates was confirmed by rDNA ITS sequence analysis. BLAST searches revealed that ITS sequences of Turkish isolates matched 99.82% (CEE-22) and 100% (CEE-211) with the Bulgarian ex-holotype strain of *D. bulgarica* (CBS 124136; GenBank GQ923854).

The pathogenicity of *D. bulgarica* strain CEE-22 obtained in this study was confirmed through an artificial inoculation experiment in the greenhouse. *Diplodia bulgarica* strain CEE-22 did cause canker lesions, and the strain proved to be highly aggressive. *Diplodia bulgarica* has previously been reported as an aggressive pathogen on *M. domestica* in Iran (Abdollahzadeh 2015, Hanifeh & al. 2017, Bari & al. 2019), India (Nabi & al. 2020), and Germany (Hinrichs-Berger & al. 2021). This is the first report of *D. bulgarica* associated with a disease of *M. domestica* from Turkey. This pathogen is considered to be an important agent causing canker on apple trees in Isparta province of Turkey.

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# *Gerhardtia foliicola*, a new record for Pakistan

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Abstract—*Gerhardtia foliicola* is reported as a new record for Pakistan. The species is characterized by a reddish brown and hygrophanous pileus, densely crowded lamellae, small sized ellipsoid basidiospores with irregular outline and occurrence on leaf litter and humus in broadleaf forests. Our phylogenetic analysis based on nuclear ribosomal internal transcribed spacer (nrITS) region clustered the Pakistani collections in the same clade with *G. foliicola* sequences from Japan. Detailed description of macro- and micro-characters, habitat, general distribution, and diagnostic features important for delimiting *G. foliicola* from closely related taxa are presented.

Key words—*Agaricales*, biodiversity, molecular phylogeny, *Lyophyllaceae*, taxonomy

## Introduction

Gerhardt (1982) published *Lyophyllum* subg. *Lyophyllopsis* Ew. Gerhardt [non *Lyophyllopsis* Sathe & J.T. Daniel (Sathe & al. 1981)], with *L. incarnatobrunneum* Ew. Bernhardt as its only species. Bon (1994) raised this *subgenus* to generic rank as *Bernhardtia* Bon stat. & nom. nov. (*Agaricales*, *Lyophyllaceae*). The type species, *G. incarnatobrunnea* (Ew. Bernhardt) Bon, is a more recent heterotypic synonym of *G. borealis* (Fr.) Contu & A. Ortega (Contu & Ortega 2002). Distinguishing characters of the genus include siderophilous granulation of basidia, minutely verrucose ornamented basidiospores, pileipellis in form of a cutis or a trichoderm, and the absence of clamp connections (Bon 1994). Vizzini &

al. (2015) emended the genus recently to emphasize smooth or verruculose basidiospores, an absence of clamp connections, and a pileipellis in form of a cutis, trichoderm, or hymeniderm.

*Gerhardtia* is a small genus (He & al. 2019) with c. seven accepted species which are distributed in Europe, New Zealand, North and South America, and China (Baroni 1981; Bon 1994; Contu & Ortega 2002; Contu & Consiglio 2004; Kalamees 2008; Mešić & Tkalčec 2009; Cooper 2014; Vizzini & al. 2015, 2017; Li & al. 2017; Matheny & al. 2017).

*Gerhardtia foliicola,* originally described from Japan as *Tricholoma foliicola* Har. Takah. (Takahashi 2001), is characterized by a saprotrophic habit and smooth basidiospores with an irregular outline (Endo & al. 2019). Species of *Tricholoma* (Fr.) Staude are characterized by tricholomatoid stature, smooth, white, and sub-globose to oblong basidiospores, and an ectomycorrhizal association with woody plants (Singer 1986), which conflict with the key characteristics of *Gerhardtia*.

Knowledge about *Gerhardtia* is limited, and previously no species of the genus has been reported from Pakistan. During various mycological expeditions, basidiomata of *G. foliicola* were collected that are presented here as a new species record for Pakistan mycobiota.

## Materials & methods

### Collection site

Specimens were collected from Kumrat valley which lies at the end of Dir Upper district, Khyber Pakhtunkhwa, Pakistan. The area falls in the alpine zone of the foothills of Hindukush where elevation ranges from 2439 to 3048 m above sea level and the annual rainfall is 52.43 cm (Stucki & Khan 1999, Siddiqui & al. 2013). The area has a dry temperate climate and is dominated by *Pinus wallichiana* A.B. Jacks. and *Cedrus deodara* (Roxb. ex D. Don) G. Don (Gillett 2001, Ahmad & Nizami 2015).

### Morphological examination

Basidiomata were collected during July–August 2019 and photographed and morphologically characterized in the field. Munsell (1975) is followed for color designations. For long term preservation, specimens were dried using an electric fan heater. Basidiomata were hand-sectioned and mounted in 5% KOH and in Congo red for contrast purposes. Melzer's reagent was used to check basidiospore amyloidity. Specimens were examined microscopically using a Meiji MX4300H compound microscope. Basidiospore, basidial, basidiole, and pileus and stipe hyphae measurements were determined from at least 20 observations at 1000 × magnification.

The abbreviation [n/l/p] refers to n (number of basidiospores) measured from l (number of basidiomata) and p (number of collections). Basidiospore dimensions are given as (a–)b–c(–d). The abbreviations used are Q = length/width ratio of spores and avQ = average Q from all basidiospores ± standard deviation (Ge & al. 2010). All specimens were deposited in the Herbarium, Institute of Botany, University of the Punjab, Lahore, Pakistan (LAH).

### DNA extraction, PCR and DNA sequencing

Genomic DNA was extracted from 5–20 mg of dried specimens using a modified CTAB method (Bruns 1995). The ITS1 +5.8S ITS2 regions were amplified in a polymerase chain reaction nuclear ribosomal DNA using primers ITS1F and ITS4 (White & al. 1990, Gardes & Bruns 1993). Amplified products were sent to TsingKe China for further purification and bidirectional sequencing.

### Phylogenetic analyses

Both forward and reverse primer reads were manually edited and assembled to generate a consensus sequence in BioEdit 7.2.5. (Hall 1999). Homology searches were carried out at NCBI webpage using the BLAST tool, and sequences with closest identity were downloaded. Closest relatives of the species and some other sequences representing the *Lyophyllaceae* were selected following phylogenetic studies (Moncalvo & al. 2000; Hofstetter & al. 2002; Cooper 2014; Bellanger & al. 2015; Vizzini & al. 2015, 2017; Latha & al. 2016; Li & al. 2017; Matheny & al. 2017; Yang & al. 2018; Endo & al. 2019). All ambiguous portions with missing data and gaps were omitted from the final matrix. Online MUSCLE tool by EMBL-EBI, (http://www.ebi.ac.uk/Tools/msa/muscle/) was used for multiple sequences alignment (Edgar 2004). The maximum likelihood tree was generated using CIPRES Science Gateway employing RaxML -HPC 2 on XSEDE with 1000 bootstrap pseudoreplicates (Miller & al. 2010). Rapid bootstrap search was performed and GTRGAMMA model was chosen. The generated tree was rooted with *Myochromella boudieri* (Kühner & Romagn.) V. Hofst. & al. (GenBank ITS: AF357047) from Switzerland as outgroup (Matheny & al. 2017). Bootstrap values ≥50% were cited on tree as significant.

## Phylogenetic results

Our sequences showed 99% identity with *Gerhardtia borealis* (KR673544) from Korea and 99% similarity with *G. foliicola* (LC458831, LC458827, LC458828, LC458834, LC458829, LC458833) from Japan (Fig. 1). A total of 39 ITS sequences were assembled for phylogenetic reconstruction. The final alignment was 732 bp, of which 317 sites were conserved, 372 were variable, 261 were parsimony informative, and 102 were singletons. Maximum likelihood analysis clustered the sequences from our Pakistani collection with Japanese *G. foliicola* with a strong bootstrap support (94%).

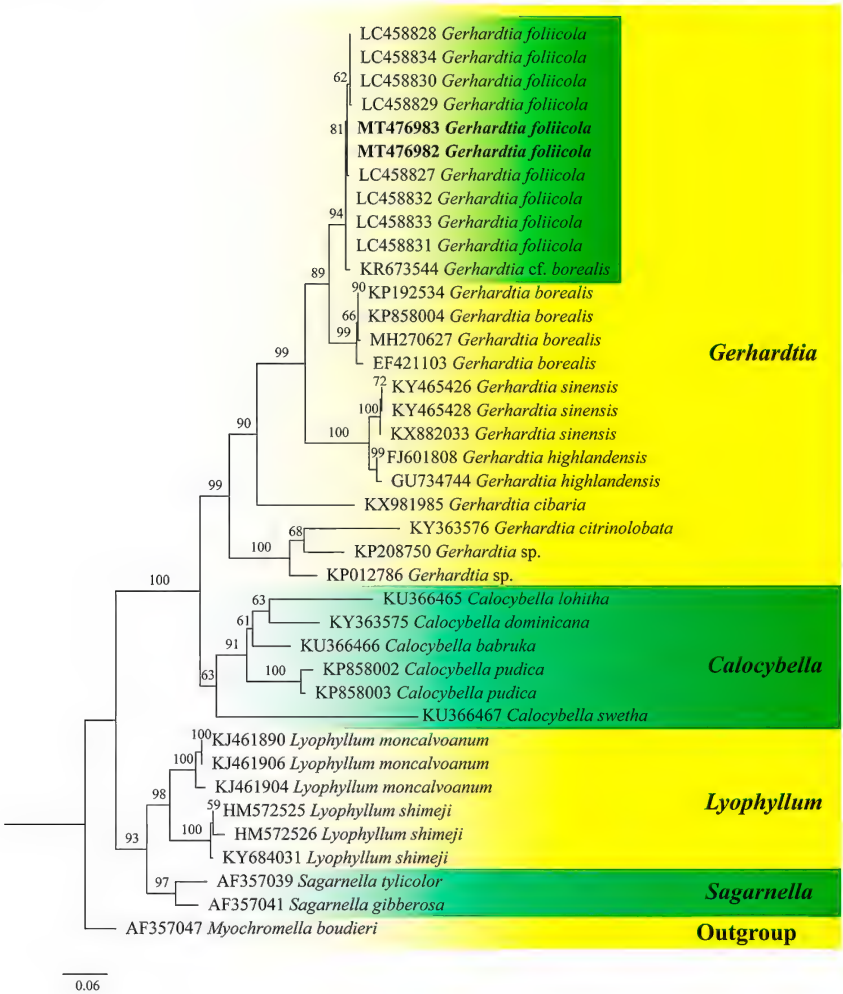


Fig. 1. Maximum Likelihood analyses of *Gerhardtia foliicola* and related taxa based on ITS sequences. The bootstrap values ≥50% given above branches. Species collected from Pakistan are highlighted in bold. The analysis included 37 nucleotide sequences.

## Taxonomy

***Gerhardtia foliicola*** (Har. Takah.) N. Endo, S. Ushijima, Nagas., Sotome, Nakagiri, & N. Maek., Mycoscience 60(6): 324. 2019. Figs 2, 3

Basidiomata thick, dry, clustered. Pileus 35–75 mm, hemispherical with rolled margins at first, later becoming convex to applanate, surface

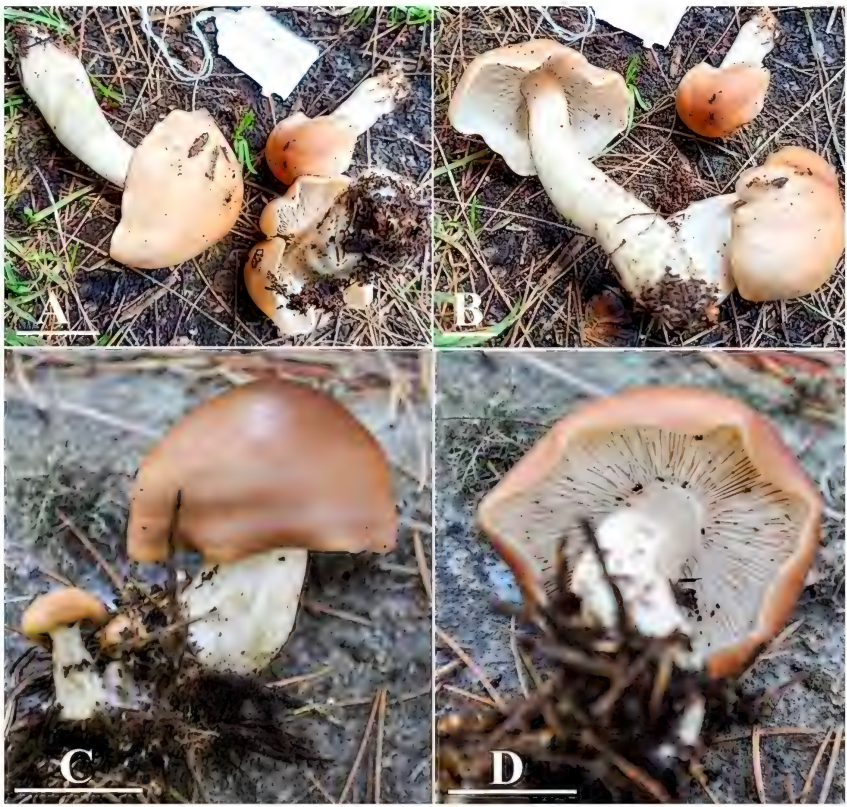


Fig. 2. *Gerhardtia foliicola*: basidiomata.
A, B = LAH 36419: C, D = LAH 36420. Scale bars = 2 cm.

glabrous, shiny when young, later dry, hygrophanous, bright reddish brown (5YR5/6), dull reddish brown (5YR5/4) to dull orange (7.5YR7/4), somewhat paler towards the margin. Lamellae white (N10), adnate to emarginate, thin, dense, lamellulae abundant. Stipe 40–120 × 10–30 mm, equal to clavate, sometimes with a bulbous base, slightly fibrillose, base covered with white tomentum, thick, solid, white (N10), light grey (5Y8/2) near the apex. Annulus and volva absent. Odor and taste not recorded.

Basidiospores [40/2/1], (4–)4.7–5.2(–5.5) × (1.7–)1.9–2.5(–2.6) µm, avl × avw = 4.6 × 2 µm, Q = 2.1–2.3, avQ = 2.2 µm, oblong to ellipsoid, smooth, sometimes with tubercles of irregular outline, thin-walled, hyaline in KOH, inamyloid. Basidia (19–)20–25(–27) × (4–)4.6–6.2(–7.6) µm, avl × avw =

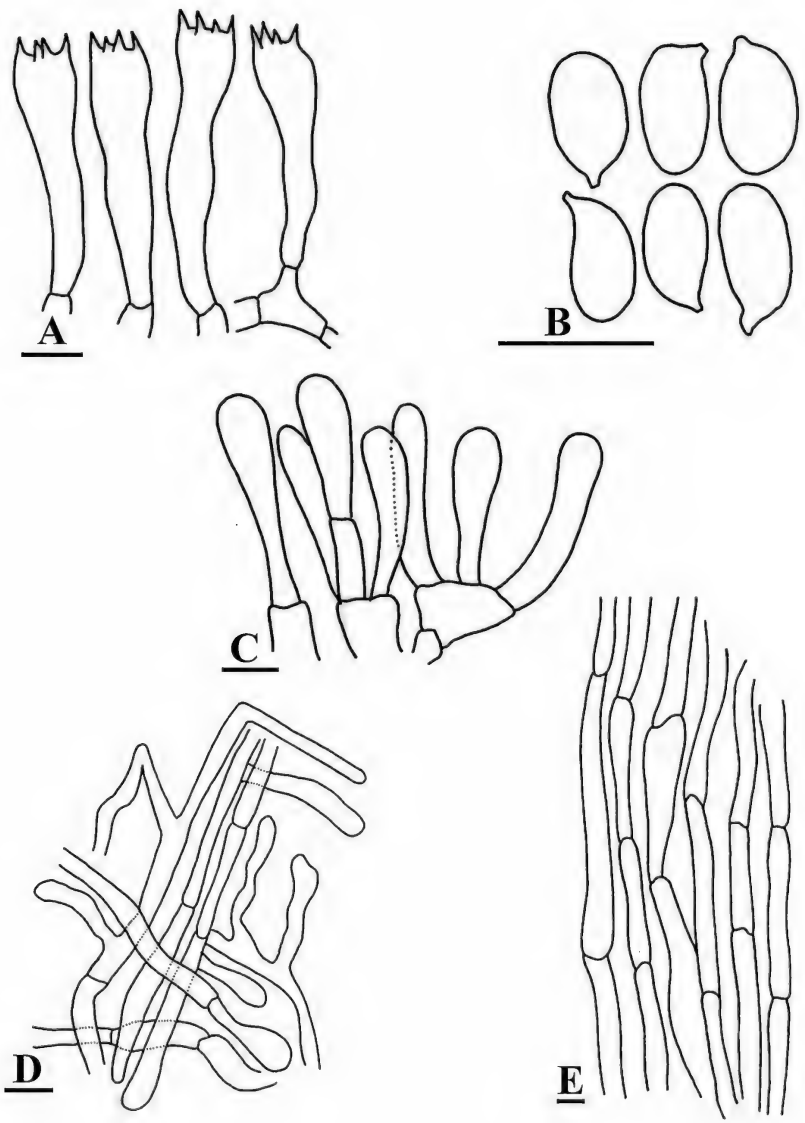


Fig. 3. *Gerhardtia foliicola* (LAH 36419, LAH 36420).
A. Basidia; B. Basidiospores; C. Basidioles; D. Pileus hyphae; E: Stipe hyphae.
Scale bars = 5 µm. Line drawings by: Aiman Izhar.

22.6 × 5.3 µm, clavate, 4-spored, hyaline in KOH, clamp connections absent. Basidioles (10.6–)13–23(–28) × (2.8–)2.9–4.7(–5) µm, avl × avw = 17.7 × 4.2 µm, clavate, basal cells septate, hyaline in KOH, clamp connections absent. Cheilocystidia and pleurocystidia absent. Pileipellis a cutis, hyphae 2–6.5 µm (avw = 4 µm) diam., thin-walled, septate, branched, ends clavate, cylindrical, hyaline in KOH. Stipitipellis a cutis of parallel hyphae, 4–11 µm (avw = 7.7 µm) diam., hyaline in KOH, frequently septate, clamp connections absent.

Habitat & distribution— Growing on leaf litter under cedars and pines. This species was originally reported from Japan and now for the first time from Khyber Pakhtunkhwa province, Pakistan.

Specimens examined—**PAKISTAN. Khyber Pakhtunkhwa: Dir (Upper) district**, Kumrat valley, 35°34′56″N 72°10′10″E, elev. 2439 m, found among pine needles, 25 July 2019, Abdul Nasir Khalid K-204 (LAH 36419; GenBank: MT476982; found on forest floor under *Cedrus deodara* and *Pinus wallichiana*, 15 August 2019, Abdul Nasir Khalid KU-19 (LAH36420; GenBank: MT476983).

## Discussion

*Gerhardtia foliicola,* originally described from Japan as *Tricholoma foliicola*, is characterized by its habit on leaf litter, a dry, glabrous and hygrophanous, reddish brown pileus, adnate and very crowded lamellae, small ellipsoid basidiospores, and hyphae lacking clamp connections (Takahashi 2001). After taxonomic reconsideration, Reschke & al. (2018) recommended transfer of *T. foliicola* to *Gerhardtia,* a recombination later supported by molecular phylogenetic analyses (Endo & al. 2019). Takahashi (2001) reported this species from a broadleaf forest dominated by *Quercus myrsinifolia* and *Q. serrata;* Endo & al. (2019) reported it as growing on humus or on leaf or bark litter of coniferous or broadleaf trees. Our findings confirm its occurrence in coniferous forests.

Our molecular phylogenetic analysis clustered the Pakistani collections in the same clade with Japanese collections of *Gerhardtia foliicola* and with a specimen from South Korea (Ulleungdo) labeled as *G.* cf. *borealis* (KR673544), which probably also represents *G. foliicola*. Phylogenetically our nrITS sequences have a single nucleotide difference at the 395 bp position when compared with the Japanese sequences. The Pakistani *G. foliicola* specimens possess a larger pileus, longer stipe, oblong to ellipsoid basidiospores, and thinner pileus hyphae contrasting slightly with the Japanese collections (characterized by a smaller pileus, shorter stipe, sub-cylindrical, ellipsoid or subfusiform basidiospores, and broader pileipellis hyphae). Geographically,

Japanese collections are recorded from temperate region and on humus and litter under conifers (*Pinus thunbergii* Parl., *P. densiflora* Siebold & Zucc., *P. parviflora* Siebold & Zucc., *Tsuga diversifolia* (Maxim.) Mast., and broadleaf trees (*Quercus* spp.), (Endo & al. 2019). The Pakistani specimens were recorded from a dry temperate climate under *Cedrus deodara* and *Pinus wallichiana*.

*Gerhardtia borealis,* a sister species to *G. foliicola* is morphologically the closest counterpart. However, it differs from *G. foliicola* by its quite distant lamellae, larger (6–8.5 × 4–5 µm) basidiospores, and growth on clay or sandy soil in coniferous forests (Contu & Consiglio 2004). This species is also phylogenetically well separated, forming a monophyletic clade that is sister to *G. foliicola* (Gerhardt 1982, Bigelow 1985, Contu & Consiglio 2004).

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*Regional annotated mycobiota new to the Mycotaxon website:*

Abstract—Mycotaxon is pleased to add a new annotated species distribution list to our 146 previously posted free-access fungae. The 39-page "Checklist of the lichens of The Reserva Florestal Adolphe Ducke in Manaus" by Aptroot, Cavalcante, Santos, Junior, Lima, and Cáceres may be downloaded from our website via http://www.mycotaxon.com/mycobiota/index.html

## SOUTH AMERICA

### Brazil

André Aptroot, Janice Gomes Cavalcante, Lidiane Alves dos Santos, Isaias Oliveira Junior, Dayane de Oliveira Lima, Marcela Eugenia da Silva Cáceres. Checklist of the lichens of The Reserva Florestal Adolphe Ducke in Manaus (Amazonas, Brazil). 39 p.

Abstract—A checklist is presented of the lichenized fungi of The Reserva Florestal Adolphe Ducke in Manaus (Amazonas, Brazil), which is one of the best studied and most accessible forest reserves in the Amazon. In total, 295 species are reported, 116 of which are newly reported here based on new sampling by the authors, 10 of which are first reports for Brazil, and 50 more are new reports to the Amazonas state. Among these, 214 are corticolous species, 81 are foliicolous and five are terricolous or on termitaria, with five species occurring both on bark and living leaves.

Key words—Amazon Forest, living leaves, diversity, termite nest, tree bark

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*Regional annotated mycobiota new to the Mycotaxon website:*

Abstract—The 13-page "First molecular-based contribution to the checklist of Lebanon macrofungi" by Sleiman, Bellanger, Richard, and Stephan may now be downloaded from Mycotaxon's mycobiota webpage. This well-documented annotated species list brings to 148 the number of free-access fungae now available on our website: http://www.mycotaxon.com/mycobiota/index.html

## MID-EAST

### Lebanon

Sandra Sleiman, Jean-Michel Bellanger, Franck Richard, Jean Stephan. First molecular-based contribution to the checklist of Lebanon macrofungi. 13 p.

Abstract—Across the Mediterranean biodiversity hotspot, our knowledge of fungal diversity is still fragmentary, and the eastern part of the basin remains poorly explored. This is particularly the case in Lebanon, where macrofungal diversity has never been subject to molecular-based investigation. A first checklist was published in 1957 and updated in 1996, based on morphological identification of specimens. In an effort to narrow this gap of knowledge, we designed a field survey in Akkar, north Lebanon in *Abies cilicica, Quercus calliprinos* and *Quercus cerris* old-growth stands, carried out between 2018 and 2019. Three sampling plots were monitored in each ecosystem. By combining morphological identification and molecular analysis of 105 collected samples, 79 species were identified, including 62 (i.e., 78.5%) new records for Lebanon, and eight putative undescribed species. Species diversity was the highest under *Quercus calliprinos* and lowest under Abies cilicica. Most species were encountered in just one ecosystem type, and only 7 were recorded in two ecosystems. These results highlight the overlooked fungal diversity in this part of the eastern Mediterranean hotspot, and the importance of Lebanese forests for biodiversity conservation. Our results also call for further investigation, in particular under endemic tree hosts, to increment the still incomplete national checklist of macrofungi in Lebanon.

Key words—basidiomycetes, forest ecosystems, mycology, Mediterranean forests, fungal diversity

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*Regional annotated mycobiota new to the Mycotaxon website:*

Abstract—The 10-page "Checklist of powdery mildew fungi (*Erysiphaceae*) in Pakistan" by Afshan, Zafar, and Khalid may now be downloaded from Mycotaxon's mycobiota webpage. This annotated species list brings to 149 the number of free-access fungae now available on our website:

http://www.mycotaxon.com/mycobiota/index.html

## ASIA

### Pakistan

N.S. Afshan, I. Zafar, A.N. Khalid. Checklist of powdery mildew fungi (*Erysiphaceae*) in Pakistan. 10 p.

Abstract—This paper presents a checklist of powdery mildews (*Erysiphaceae*) in Pakistan together with their known host plants. A total of 42 taxa belonging to 10 genera are included here. A host index is also provided..

Key words—angiosperms, *Ascomycota,* biotrophic parasites, *Erysiphe*

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*Regional annotated mycobiota new to the Mycotaxon website:*

Abstract—Mycotaxons pleased to add our 150th annotated species distribution list to our previously posted free-access fungae. The extraordinarily well-documented 57-page "Aphyllophoroid fungi (*Basidiomycota*) of Chile: An annotated checklist" by Riquelme & Rajchenberg may be downloaded from our website:
via http://www.mycotaxon.com/mycobiota/index.html

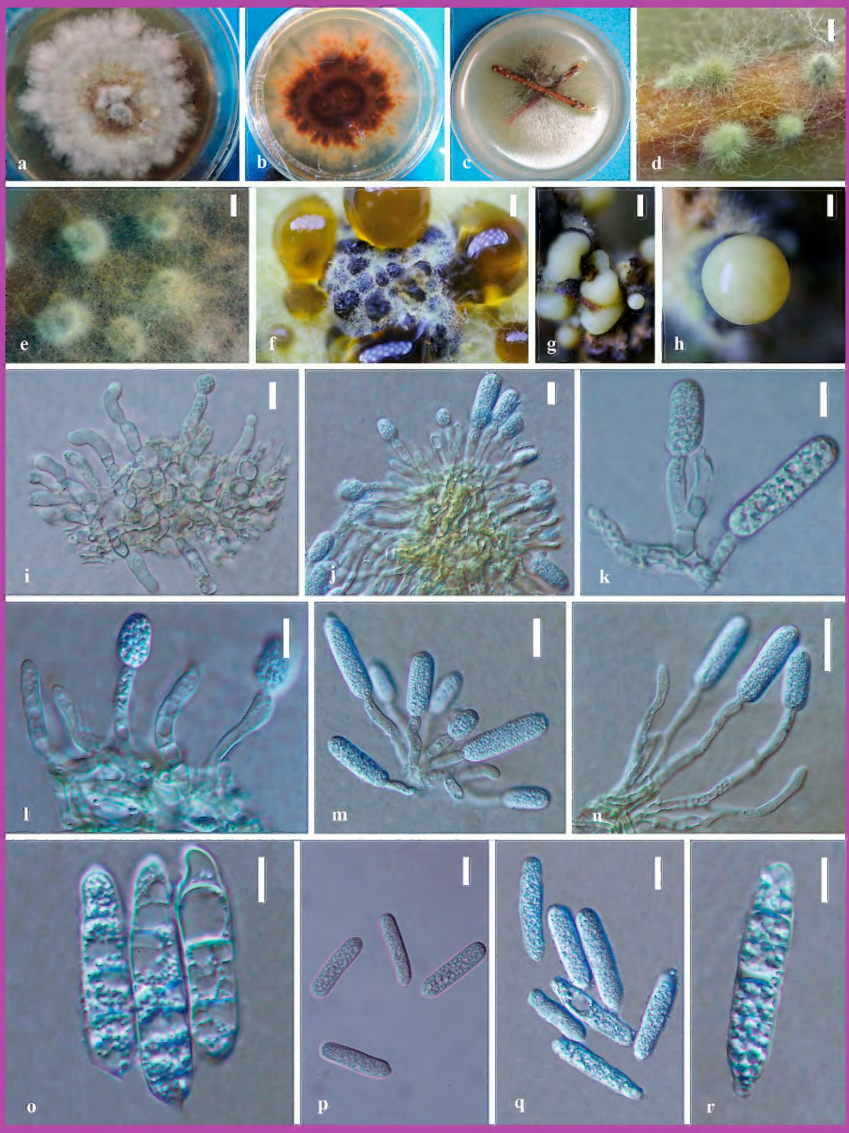
## SOUTH AMERICA

### Chile

Cristian Riquelme & Mario Rajchenberg. Aphyllophoroid fungi (*Basidiomycota*) of Chile: An annotated checklist. 57 p.

Abstract—We critically reviewed the aphyllophoroid fungal species recorded from Chile on the basis of literature reviews from 1828 to 2020. A total of 345 species names were distributed in the following categories: accepted taxa (236), uncertain taxa (83), taxa inferred by molecular analysis only (12), and excluded taxa (14). For each accepted species, information on the species name, its first record from Chile, the typification data, substrate, distribution, and remarks are presented. The distribution of accepted taxa shows a major recording from the Valdivian Forest province. We found that 61 (25.85%) of the accepted taxa were originally described from Chile. *Nothofagus* was the genus that hosted the highest number of fungal species.

Key words—aphyllophorales, coralloid fungi, corticioid fungi, polypores




*Pezicula endophytica* sp. nov.
(Ma & al.— Fig. 2, p. 571)